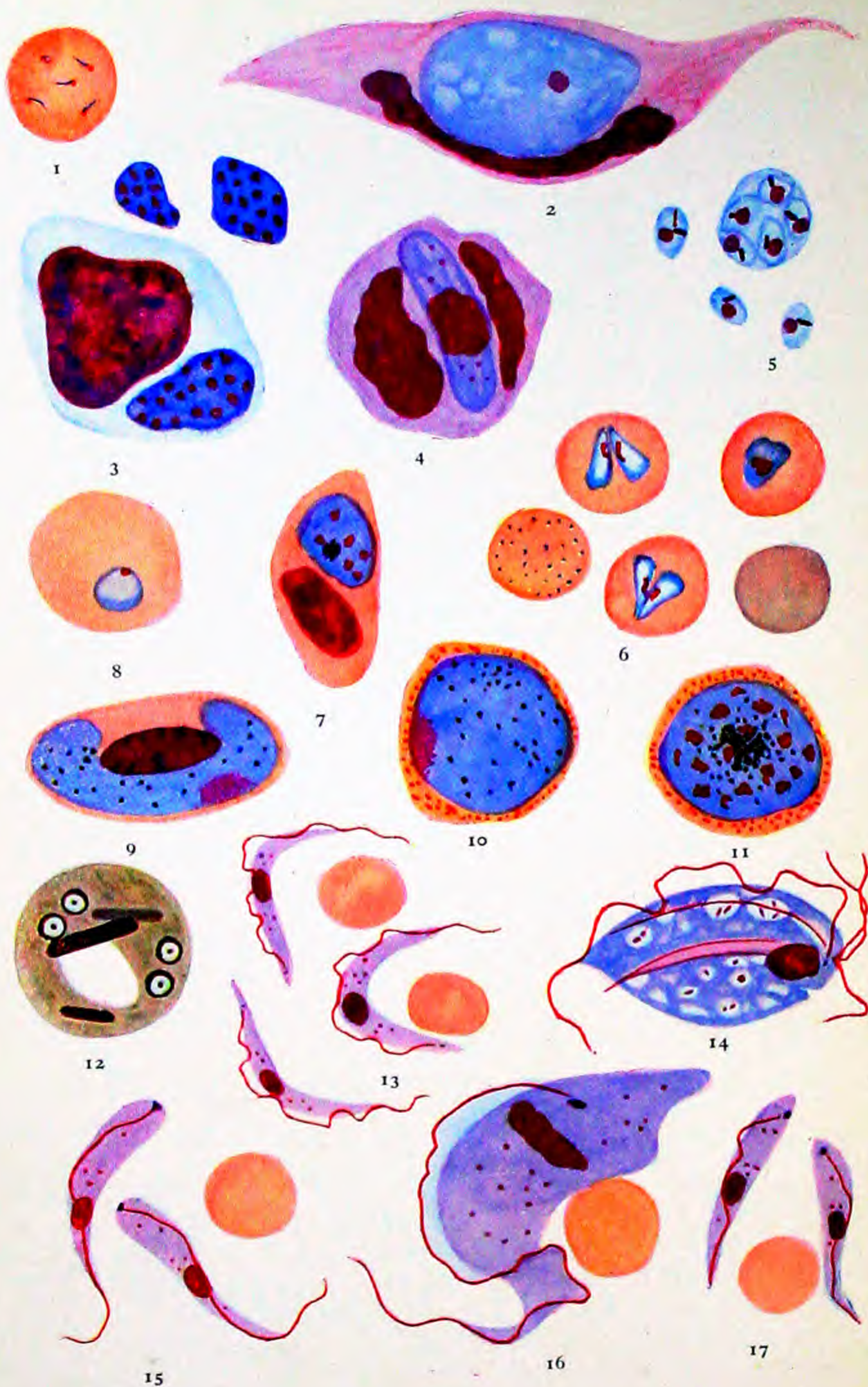


VETERINARY PROTOZOOLOGY



PROTOZOAN PARASITES IN STAINED PREPARATION
[W. COOPER, (pinx)]

1. *Theileria parva* in erythrocytes of ox
2. *Leucocytozoon neavei* from guinea-fowl
3. *Theileria parva*. Blue bodies
4. *Hepatozoon canis*
5. *Leishmania donovani*
6. *Babesia canis* in blood of dog
7. Avian malaria (developing schizont)
8. Monkey malaria (young schizont)
9. *Hæmoproteus columbæ*
10. Monkey malaria (macrogametocyte)
11. Monkey malaria (developing schizont)
12. *Entamoeba histolytica* (cyst)
13. *Trypanosoma equiperdum*
14. *Trichomonas*
15. *Trypanosoma vivax*
16. *Trypanosoma theileri*
17. *Trypanosoma congolense*

VETERINARY PROTOZOOLOGY

BY

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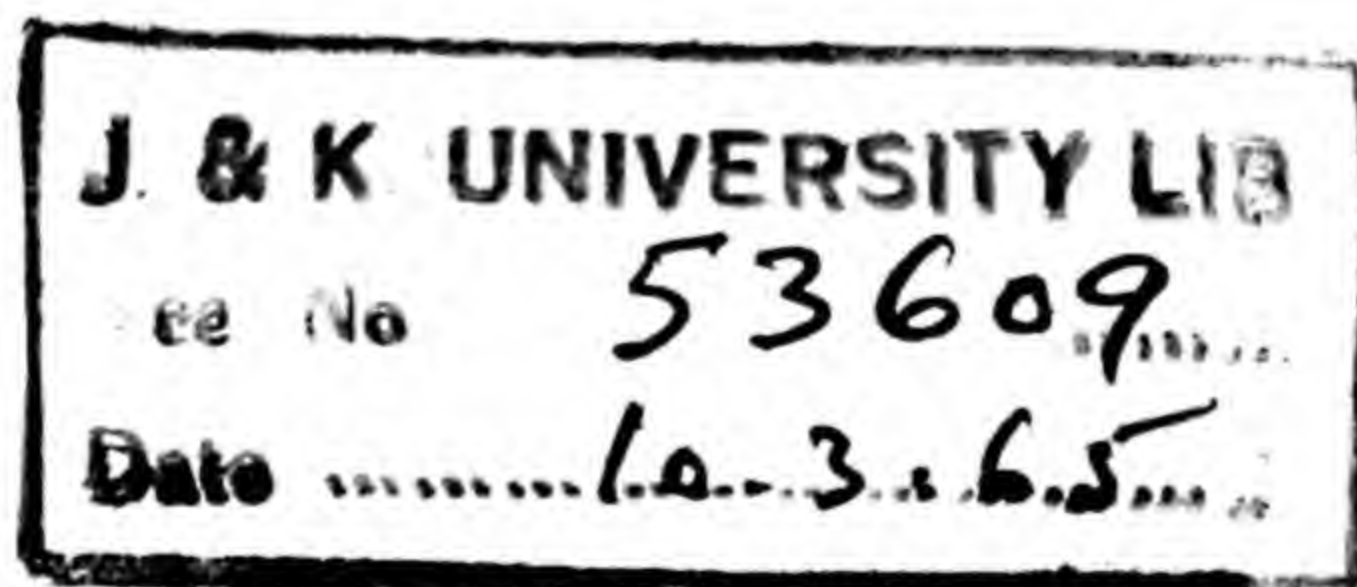
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PREFACE

THE information available to veterinary surgeons on the protozoan parasites of animals, and their relationship to animal diseases, has up to the present been scanty and unsatisfactory. The subject has been dealt with in books devoted to veterinary bacteriology and parasitology, and the sections on protozoology have been included mainly owing to the absence of any comprehensive text-book on the pathogenic protozoa themselves, and have been purely a secondary feature, dealt with mainly from an academic outlook. Text-books which claim to cover medical and veterinary protozoology are available, but these have been written primarily from the medical aspect, and the information on the parasites of domestic animals is sometimes inaccurate, and often misleading as regards their practical importance to animal husbandry. Original articles are scattered through veterinary, medical and agricultural periodicals, and are often difficult to obtain for consultation.

This book has been written in the hope that it may be of value to veterinary students, and to workers in the tropics, where protozoan diseases are the principal problems of the veterinary surgeon, and the chief obstacle to stock improvement. It is also hoped that it may stimulate interest and lead to research work, which will benefit both veterinary and human medicine. It seems probable that even in temperate climates protozoa will be found to be responsible for more disease than has been ascribed to them. Toxoplasms, for instance, are known to cause disease in man but though they have been recorded in several species of domestic animals little is known as to their clinical importance.

I have to thank the Zoology Department of Edinburgh University and the Parasitology Department of the London School of Hygiene and Tropical Medicine for giving me accommodation at various times to facilitate the preparation of this book, and Colonel H. E. Shortt, Professor of Medical Protozoology of the latter Institute, for allowing Mr W. Cooper (the artist who prepared the drawing for the frontispiece) to help me with the compilation of the chapter on technique. I also have to thank

Mr J. Carmichael, D.Sc., M.R.C.V.S. ; Mr S. J. Gilbert, M.R.C.V.S., and Mr J. D. Campbell, F.R.C.V.S., for their criticisms of early drafts of the book, and the last for the photograph of blackhead infection of the liver. I also have to thank Mr C. Horton-Smith for the photograph of *E. tenella* infection of the cæca of a chicken.

PREFACE TO THE THIRD EDITION

THE preparation of a third edition of *Veterinary Protozoology* has been made the occasion for a further thorough revision. In attempting to produce, in a volume of moderate size, an account which while concise yet gives due regard to the considerable amount of new information available, the author must necessarily give particular consideration to the relative importance of his different subjects. Thus, a strict adherence to the title of the book would suggest that the section dealing with human malaria should be discarded. It was felt, however, that the subject is of such outstanding general interest that a brief account should be retained although the revision and enlargement of the section, which would certainly be required by recent advances in knowledge, was not justified.

Serious consideration was given also to the group of parasites described as "Parasites of uncertain classification". Over recent years there has been much discussion as to the systemic position of these organisms and in some instances, at least, it is fairly clear that they cannot accurately be described as Protozoa. In a recent review Levine (1961) has concluded that *Besnoitia*, *Encephalitozoon*, *Sarcocystis* and *Toxoplasma* are fairly closely related Protozoa but that *Anaplasma*, *Eperythrozoon*, *Grahamella* and *Hæmobartonella* belong to the *Rickettsiales* while *Bartonella* is a bacterium. It has been decided nevertheless to retain sections on all these organisms in the present volume if only because their epidemiology and chemotherapy have traditionally been considered along with those of the Protozoa proper and might not be found elsewhere.

As with previous editions of *Veterinary Protozoology* particular attention has been paid to the epidemiology and control of

disease and there are substantial sections dealing with chemotherapy. These, it is hoped, will be useful to veterinarians working under field conditions where full library facilities may not be available. In the compilation of the new edition the author, as on previous occasions is greatly indebted to a number of colleagues both in Britain and abroad. Particular reference must be made to Dr J. K. A. Beverley, of Sheffield University, to Dr Savage of the University of Manitoba and to their publishers for help with the illustrations, to Messrs T. M. Leach and Walter Petana of the West African Institute for Trypanosomiasis Research at Kaduna for an excellent series of photographs of *Trypanosoma* and *Glossina* and to Mr S. F. M. Davies and Dr L. P. Joyner for their agreement to the inclusion of copyright material from the recent publication *Coccidiosis*.

It is a pleasure also to acknowledge our thanks to Dr Hoare and to Messrs Baillière, Tindall and Cox Ltd. for their permission to reproduce figure 2 from their *Handbook of Medical Protozoology*. Figures 25 and 35 are reproduced from Wenyon: *Protozoology*, by permission of the same publishers.

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CHAPTER I

INTRODUCTION

THE NATURE OF PROTOZOA: CLASSIFICATION

THE *Protozoa* are unicellular members of the Animal Kingdom. The fact that all their metabolic processes occur within the confines of a single cell, while those of the *Metazoa* appear to need the much more complicated organisation of tissues and specialised organs, makes them of particular interest for the study of the fundamentals of biology.

In general, the protozoa can be distinguished from the analogous group of unicellular plants (the *Protophyta*) on the grounds of morphology, the way they obtain their food and the way in which they reproduce. Protozoa possess a well defined nucleus, whereas in the protophyta the nuclear material is scattered throughout the cytoplasm as granules which may often be difficult to detect. Plant cells are nearly always bounded by a more or less rigid wall made of cellulose whereas animal cells are bounded by a delicate non-rigid membrane, the *periplast*; for this reason bacteria of a single species show considerable uniformity in shape and size while protozoa of a single species may vary considerably in both these characteristics. Plant cells usually obtain their food through *holophytic* means, *i.e.* they use the green pigment chlorophyll, carried in *chromatophores*, to synthesize sugars. Animal cells, by contrast, are typically *holozoic* and require ready-made organic material for food. Reproduction in both plant and animal cells may be by binary fission but as a rule protozoa divide through the longitudinal axis whereas protophyta divide transversely to the long axis of the body.

These are differences which are generally applicable but there are plenty of exceptions, as to all biological rules. For example, among the protozoa, most *Ciliates* divide transversely and some species of *Mastigophora* belie their animal nature by bearing chromatophores. In general, however, it is fairly easy to distinguish plant from animal cells.

THE HISTORY OF PROTOZOOLOGY

Because the majority of protozoa are too small to be seen with the naked eye their study had to await the discovery of the microscope. In the late seventeenth century Antoni van Leeuwenhoek, with the aid of a simple microscope, was able to describe several free-living protozoa and later a parasite, probably *Giardia intestinalis*, with which he himself was infected.

Parasites of veterinary importance were discovered at a very early date for Leeuwenhoek appears to have discovered the oocysts of *Eimeria stiedæ* in the gall bladder of a rabbit as early as 1674 ; but it was not till nearly 200 years later that in 1865, Lindemann recognised the true nature of the parasite. In 1879 Leuckart created the class of *Sporozoa*. The systematic study of the Eimeriæ of fowls, another landmark in the field of veterinary Protozoology, was accomplished by Tyzzer in 1927 to 1929. This was largely responsible for the modern conception of coccidiosis. In 1880 Laveran discovered the malarial parasite in human blood and in 1888 Babes described piroplasms in the blood of cattle. *Babesia bigemina* was accurately described by Smith and Kilborne in America in 1893. They also established that the parasite was transmitted by a tick, *Boophilus annulatus*, and they showed that the parasite survived in the eggs of ticks, infection being transmitted by the next generation. In 1898 Ross showed that *Plasmodium relictum* of birds was transmitted by the mosquito *Culex fatigans*. From this followed the discovery of the vector of human malaria by Bastianelli, Bignami and Grassi in the same year. In 1898 Koch first saw *Theileria parva* in the blood of cattle in East Africa, although he considered it to be a stage in the development of *Babesia bigemina*. The organism was not named till 1904 by Theiler, while the transmission by *Rhipicephalus appendiculatus* was demonstrated by Lounsbury (1902-1904).

Following the discovery of *Giardia intestinalis* by Leeuwenhoek, Davaine recorded *Trichomonas* and *Chilomastix* in the excreta of cholera patients in 1854. The first seriously pathogenic intestinal flagellate to be recorded was *Histomonas meleagridis*, the causal organism of "Blackhead" in turkeys, described by Theobald Smith in 1895. *Trichomonas vaginalis* of the human urino-genital tract was described by Donné in 1837. In 1841

Valentin observed a flagellate in the blood of a salmon and in 1842 Gluge and Gruby discovered a trypanosome of the frog. In 1878 Lewis observed *Trypanosoma lewisi* of the rat and in 1881 Griffith Evans discovered the first pathogenic blood flagellate, *Trypanosoma evansi*, the causal organism of *surra*, a disease of camels and horses. In 1893 Bruce, investigating trypanosomiasis of stock in Africa, elucidated the cyclical development of *Trypanosoma brucei* in the tsetse fly *Glossina pallidipes*. *Entamoeba gingivalis* of the mouth was recorded by Gros in 1849 and the first pathogenic amœba, *Entamoeba histolytica* by Lösch in 1875 in Russia.

Recent years have seen a marked advance in the study of Protozoology, largely as the result of an increasing appreciation of the outstanding importance of diseases such as malaria in man and trypanosomiasis in domestic animals.

THE MORPHOLOGY OF PROTOZOA

Throughout the Phylum there is a great variation in size but the parasitic protozoa are mostly small. Sometimes the organisms may occur as colonies or cysts which are visible to the naked eye. Most protozoa are without symmetry, although in a few, e.g. *Hexamita* there is bilateral symmetry. Fundamentally, the body is composed of protoplasm which is always differentiated into *nucleus* and *cytoplasm*.

The nucleus

Most protozoa contain a single nucleus although some may possess two or more during at least part of the life-cycle. Organisms such as *Balantidium* have two nuclei—a larger *macronucleus* and a smaller *micronucleus*. Nuclei are broadly classified as *vesicular* and *compact*. The *vesicular* nucleus usually has an intranuclear body, which if composed of chromatin material is called the *karyosome*. The *compact* type of nucleus contains a large amount of chromatin substance. The macronucleus of the *Ciliata* is nearly always of this kind.

The cytoplasm

The extranuclear part of the protozoon is the cytoplasm. This is extremely variable in character and may be pigmented

with chromatophores or with the symbiotic chlorophyll-containing algæ previously mentioned. The cytoplasm may in turn be differentiated into a cortical zone (*ectoplasm*) and an internal *endoplasm*. The ectoplasm is usually bounded by the protective cuticle—the *pellicle* or *periplast*. Sometimes the body is further protected by a shell (the free-living *Foraminifera*), or, as a special protection against adverse conditions during the phase of the life-cycle which is outside the body of the host, by a stout spore wall (*Eimeria*).

Within the cytoplasm may be special structures which closely simulate the more complex organs of the *Metazoa*. In the simplest types of protozoa the food is taken in through the general surface of the body but in some there may be a *cytostome* (mouth) which is set at the bottom of a depression or channel known as the *peristome* and through which food is collected as the result of the movement of special tracts of cilia.

The rigidity of the ectoplasm is the main factor in retaining the shape of the organism. There are, however, in some organisms, fibrils which support such structures as the cytostome. Some protozoa possess a stiff rod, the *axostyle* which passes through the body and may protrude at the posterior end. The differentiation of an anterior from a posterior end is possible in motile organisms. If a cytostome exists it is considered to open on the ventral surface.

Contractile vacuoles are found in most free-living protozoa but parasitic *Sarcodina* and *Mastigophora* do not usually have them. The organ is present in parasitic *Ciliata* but not in *Sporozoa*.

LOCOMOTION IN THE PROTOZOA

Locomotion is by *pseudopodia*, *flagella* or *cilia*.

Pseudopodia

These are temporary projections of part of the cytoplasm. They are characteristic of the *Sarcodina*. They have been classified according to their form as

(a) *Lobopodia*—extension of the ectoplasm with an inflow of endoplasm as occurs in *Amœba proteus*.

(b) *Filopodia*—formed as filamentous projections composed almost entirely of ectoplasm.

(c) *Rhizopodia*—also filamentous but branching and anastomosing.

(d) *Axopodia*—Semi-permanent structures composed of axial rods and a cytoplasmic envelope. These are found in free-living forms such as *Heliozoa*.

Flagella

The flagellum is a filamentous extension of the cytoplasm and is usually very fine and highly motile. Under the microscope it is very much more easy to see the waves of motion set up by the flagellum than the organ itself. The flagellum consists of an elastic axial filament (the *axoneme*) and a contractile cytoplasmic sheath surrounding it for a part or the whole of its length. At the base of the flagellum are two darkly staining structures—the *kinetoplast* (*kinetonucleus* or *parabasal body*) and the *blepharoplast* (*basal granule*). Some authors have used the term “kinetoplast” for the compound structure comprising the parabasal body and the blepharoplast. The blepharoplast is often a conspicuous compact granule. Very occasionally it may be exceedingly small or absent (*Trypanosoma equinum*). The flagellum is most often inserted near the anterior end of the body and is directed forwards. There may be a trailing flagellum which is directed posteriorly. Occasionally, there may be several flagella (e.g. *Trichomonas*). In some parasitic *Mastigophora*, e.g. *Trypanosoma*, there is a delicate membrane extending along the side of the body with the flagellum running along its outer margin. This is the *undulating membrane*. It assists in locomotion.

Cilia

These are the organs of locomotion found among the *Ciliata*. In addition to being concerned in locomotion they may aid in the ingestion of food and they often serve as tactile organs. Cilia are fine, comparatively short processes of ectoplasm. They vary in length and may be arranged in definite tracts—in longitudinal, oblique or spiral rows. Dense areas of cilia are sometimes described as *ciliary fields* or *ciliary zones*. Sometimes zones of cilia become fused into a plate called a *membranella* which, if

occurring on the margin of the peristome, forms the adoral zone which assists in the collection of food.

THE PHYSIOLOGY OF PROTOZOA

Nutrition

Nearly all protozoa obtain their nutrition by *holozoic* means, *i.e.* they use already elaborated animal or plant tissues for food. A few protozoa are able to synthesize sugars from carbon-dioxide, by the use of chlorophyll, in the same way as plants. In many instances, however, green pigmented protozoa are really in symbiotic association with algæ.

Free-living protozoa absorb food by a variety of means. In the simplest instances, the food is engulfed by an enveloping flowing movement of the protoplasm, as in *Amæba*, or by the formation of special enfolding pseudopodia. In other instances, food is taken into the body through a specialised *cytostome* or mouth. When the food particles have been engulfed they are usually digested in food vacuoles.

Parasitic protozoa absorb the digested or decomposed substance of the host. Sometimes it appears that the host tissues are digested by enzymic action before absorption. The parasitic protozoa such as *Entamæba histolytica* and *Balantidium coli* actually engulf host tissue cells.

Recent investigations into the physiology of protozoa have greatly assisted the study of chemotherapy. It has been found, for example, that the sulphonamides exert their powerful effect through interference with a metabolic chain which involves para-amino-benzoic acid. In a similar way anti-malarial drugs such as pyrimethamine are believed to interfere with the metabolism of folic acid. Both para-amino-benzoic acid and folic acid are involved in the synthesis of nucleic acid, the formation of which is particularly necessary to the parasite during the very active process of schizogony. In practice, it is found that chemotherapy is often most effective against the schizont.

Respiration

Like every other living thing the protozoon must respire. Most free-living and some parasitic protozoa utilise free molecular

oxygen but many parasites are able to derive their oxygen by splitting complex oxygen-containing substances in the tissue of the host. They, in fact, behave as anærobes. Often particular developmental stages of the parasite may have particular oxygen requirements. Thus, the oocysts of *Eimeria* require very well oxygenated conditions in order to sporulate.

Excretion and secretion

The breakdown products of the metabolism of protozoa, the water, carbon-dioxide and nitrogenous material, pass out by diffusion through the general surface of the body or by discharge through the contractile vacuole. This accumulates waste material for some time before discharge. It will be remembered that parasitic protozoa do not usually have a contractile vacuole and it has been reasoned that this absence indicates that its primary function is the excretion of water. Parasitic protozoa, unlike the free-living forms, live in an approximately isotonic medium. As a result they do not require to dispose of large quantities of surplus fluid.

The crystals and granules of various kinds which are seen in the bodies of protozoa are considered to represent the katabolic products of metabolism. In particular cases the origin of granules may be clear. Thus melanin granules in *Hæmosporidia* can be assumed to be derived from the hæmoglobin of destroyed erythrocytes.

Reproduction

The *trophozoite* is the ordinary vegetative form of the parasite which feeds and grows, eventually to divide to form daughter individuals by which the process is repeated.

In nearly all instances the reproduction of protozoa is initiated by nuclear division which is accompanied by division of the extra-nuclear "organs" such as chromatophores.

Binary fission, i.e. division of the trophozoite into approximately equal parts by fission through the long axis of the body is very commonly seen among protozoa. In *multiple fission* the body divides into a number of new individuals, this being accomplished either by repeated binary fission or by budding (*gemination*), i.e. the formation of one or more individuals from the

parent. *Schizogony* is regarded as a special form of multiple fission in which there is an initial multiple division of the nucleus followed by the budding-off of *merozoites* which are formed by a portion of cytoplasm surrounding each of the nuclei. The aggregation of nuclei and cytoplasm before the release of the merozoites is known as the *schizont*.

During the life-cycle of a protozoon there may be several successive schizogonous divisions but ultimately there will be a sexual process. Throughout the Phylum the process varies. Sometimes two individuals come together and exchange some nuclear material, after which process of *conjugation* they again separate. Otherwise there may be sexual reproduction involving the fusion of two similar sexual cells (*isogametes*) or the fusion of two dissimilar cells (*anisogametes*).

When there is a marked difference between the sexual cells, the larger (female) cell is known as the *macrogamete* and the smaller cells are known as *microgametes*. Macrogametes and microgametes are derived respectively from *macrogametocytes* and *microgametocytes*. The fusion of the two sexual cells results in the formation of a *zygote*.

THE ECOLOGY OF PARASITIC PROTOZOA

Free-living animals show adaptation to the particular ecological *niche* in which they live in association with other animals and plants. The study of parasitism is merely the study of a particular branch of ecology, the habitat of the parasite being the tissues of the host.

Parasitism is an association between two organisms in which one (the parasite) lives at the expense of the other (the host). For convenience, parasitism is distinguished from *commensalism*, in which two organisms live together without evident benefit or loss one to the other, and from *symbiosis* which is a relationship of mutual advantage. As with all biological relationships it is often difficult clearly to separate one form of association from another ; commensalism merges into symbiosis and parasitism without a clear distinction. When considering the evolution of the parasitic habit it may be believed that the protozoa were originally saprophytic, living in the gut of the potential host and

eventually taking on a symbiotic relationship (as with the bacteria in the gut of herbivores). From the symbiotic to the parasitic habit may be a very short step.

In common with all parasites the protozoa have adopted special means to ensure dispersal and to overcome adverse environmental conditions while they are passing from one host to another. Sometimes this involves the interposing of an insect (blood-sucking) vector between two mammalian hosts. Often there is a resistant cyst which may be produced by the trophozoite (*Entamoeba histolytica*) or by the zygote (*Eimeria* spp.). The encysted form may be able to survive adverse conditions for very considerable periods of time. Sometimes, as with some free-living ciliates, the formation of the cyst is combined with the reproductive process, and division within the cyst is followed by the release of the daughter individuals. With *Eimeria* the maturation of the cyst (the *oocyst*) outside the body of the host involves the division into *sporocysts* and *sporozoites*.

During the development of the spore of *Myxosporidia* and *Actinomyxidia*, *polar filaments* are formed. These are believed to act as temporary anchoring organs of the spore while it is becoming established in the gut of the host. Their presence or absence is used in the classification of the *Sporozoa*.

THE BALANCE BETWEEN HOST AND PARASITE

The process of evolution has inevitably resulted in the production of parasites which are well adapted to their environments, *i.e.* to their usual hosts. Because the environment supplied by one particular species of host-animal will differ from that offered by another it follows that some degree of *host-specificity* is likely to arise. Parasites evince host-specificity in varying degree but in some instances, *e.g.* the genus *Eimeria*, a particular species may be not only host-specific but also organ-specific. Thus *Eimeria tenella* is confined to the cæcum of the common fowl.

The relationship between host and parasite involves a compromise between, on the one hand, the tendency of the host to destroy the parasite and on the other, the tendency for the parasite to overwhelm the host. Such a relationship is never static and the delicate balance may at any time be destroyed. Either one of

the two partners—the host and the parasite—may then suppress the other. The veterinary protozoologist is, of course, concerned primarily with the circumstances which lead to the parasite gaining ascendancy over the host.

After initial infection, the following may occur :

(1) *Elimination of the parasite.* This may occur if the host is unsuitable as the result of natural or of acquired resistance.

(2) *Death of the host.* This may follow the entrance of the parasite into an abnormal host. It may, however, result from hyper-infection.

(3) *Establishment of the parasite with completion of its developmental history.* Completion of the life-cycle may be followed by the elimination of the parasite and with the establishment of a variable degree of resistance to reinfection. This resistance is rarely as absolute as that which arises from many bacterial infections but it is often sufficient to protect the host from clinical disease. The exact mechanism by which resistance is attained is not usually understood. Sometimes the young animal may be relatively resistant (*e.g.* the bovine and *Babesia bovis*). More often there is apparently an age resistance (*e.g.* the turkey infected with *Histomonas meleagridis*) but often it is difficult to be sure that an apparent age resistance is not really the result of previous infection. The demonstration of specific antibodies in the sera of resistant animals has been made with, for example, several species of *Trypanosoma*, but not, for example, with species of the genus *Theileria*. With *Trichomonas foetus* there is evidence both of humoral and of local antibody production.

Under other circumstances the parasites may not be eliminated from the host but are retained, usually in moderate numbers ; sometimes, as with the trypanosomes in the African wild game, in a relationship which is predominantly benign and not easily affected by changes in the host and sometimes, as with *Trypanosoma congolense* in African cattle, in a state of premunity. *Premunity* is considered to indicate a low-grade persistent infection, the existence of which precludes further infection but which may be subject to change as the result of changes in the circumstances of the host. Stress factors, such as intercurrent disease, are believed to cause a premune infection to change its character and lead to virulent disease.

THE WAY IN WHICH PROTOZOA CAUSE DISEASE

The degree of disturbance to the host varies very greatly with the species of parasite and with the natural or acquired resistance of the host. Often, there may appear to be a considerable variation in pathogenicity according to the geographical area in which the parasite is found. This may be related to the existence of different geographical races of the parasite, or host.

Damage to the host may result from the parasite utilising necessary supplies of food or other materials or through specific effects such as an interference with the calcium and phosphorus metabolism. Damage may be caused through destruction of erythrocytes, or particular organs and tissues may be affected. Sometimes the pathogenicity arises from a general toxæmia.

With the coccidia there is no evidence of a general toxæmia but specific organs may be grossly affected. Thus, with the intestinal species of *Eimeria* the process of asexual multiplication (schizogony) can cause excessive damage to the intestinal epithelium which appears to lose its ordinary ability to absorb food material. Much of the pathogenic effect of such an infection may not become apparent until some time after the parasite has completed its cycle of development. With *E. tenella*, by contrast, death can nearly always be ascribed to the copious hæmorrhage which follows the simultaneous maturation of the large second generation schizonts. Tissue damage, which is subepithelial, leads to rupture of innumerable small blood vessels and the bird dies from loss of blood. Specific organ damage may follow the growth of a parasite such as *Entamæba histolytica* which primarily parasitises the glands of the colon in man. The first evidence of infection is the appearance of slightly raised nodules which develop into abscesses. This may be followed by ulceration and the complete disruption of areas of the wall of the colon. Similar pathological changes may follow infection with *Balantidium coli*.

Following infection with the species of *Plasmodium* which cause malaria, there may be enormous enlargement of the spleen, the blood becomes thin and watery and the erythrocytes decrease very greatly in numbers. The mononuclear cells increase in

numbers. The hæmopoietic tissue becomes hypertrophied. With *Plasmodium falciparum*, as with some species of *Trypanosoma* and *Babesia*, the blood capillaries of the brain, and sometimes the spleen and other viscera, may become blocked by infected erythrocytes.

With human sleeping sickness, caused by trypanosomes, the principal lesions are in the lymphatic glands and the central nervous system. *T. gambiense* causes enlargement of the glands and spleen which is followed by changes in the meninges and by an increase in the cerebro-spinal fluid. Central nervous symptoms are not so characteristic of the similar disease caused by trypanosomes in cattle in Africa.

Following infection with *Leishmania donovani* there is a large increase in the numbers of macrophages and mononuclear cells and also an extreme enlargement of the spleen.

With all protozoal infections acute disease usually occurs in the early stages of infection while the parasites are multiplying rapidly. If the host survives the period of early invasion the rate of multiplication of the parasite is likely to decrease and symptoms will abate. Sometimes, however, as has already been indicated, the gross damage caused early in the disease may become increasingly apparent as the disease takes on a chronic character. Death may occur although parasites are no longer present.

THE CLASSIFICATION OF PROTOZOA

Originally the amœbæ were regarded as the most primitive forms of protozoa but it is now considered that the flagellates, which include some holophytic forms which obtain their food in the same way as plants, represent the ancestral forms. By contrast the ciliates are the most highly organised of all protozoa. The phylum is divided into four main groups, the classes *Mastigophora*, *Sarcodina*, *Sporozoa* and *Ciliata*.

A true appreciation of the relationships between the numerous different species of protozoa would necessitate an examination of the classification which is beyond the scope of this book. The following, which is based on the classification of Kudo (1954) indicates only those protozoa which are of direct interest to the veterinarian.

CLASS (1) **THE MASTIGOPHORA**—characterised by a trophozoite with one or more flagella.

SUB-CLASS—Zoomastigina (no chromatophores)

ORDER (1) *Protomonadina* (one to two flagella)

Family *TRYPANOSOMIDÆ* (one flagellum ; no collar ; parasitic)

Genera : *Trypanosoma*, *Leishmania*

ORDER (2) *Rhizomastigina* (pseudopodia as well as flagella)

Family *MASTIGAMÆBIDÆ* (one to three flagella, rarely four)

Genus : *Histomonas*

ORDER (3) *Polymastigina* (three to eight flagella)

Family *TRICHOMONADIDÆ* (with axostyle and undulating membrane)

Genus : *Trichomonas*

Family *HEXAMITIDÆ* (binucleate with bilateral symmetry)

Genera : *Hexamita*, *Giardia*

CLASS (2) **THE SARCODINA**—(Protozoa with pseudopodia for locomotion)

SUB-CLASS—Rhizopoda (with lobopodia, rhizopodia, or filopodia)

ORDER *Amœbina* (with lobopodia)

Family *ENDAMÆBIDÆ* (parasitic, only amœboid stage in life history)

Genus : *Entamœba*

CLASS (3) **THE SPOROZOA**—(Protozoa with no cell organs of locomotion ; parasitic)

SUB-CLASS—Acnidosporidia (Spore with membrane ; one sporozoite ; no polar filament)

ORDER *Sarcosporidia* (muscle parasites ; form Miescher's tubes)

N.B.—The precise status of *Sarcocystis* (the only genus) is in doubt.

SUB-CLASS—Telosporidia (spore with or without membrane ; one or many sporozoites ; no polar filament)

ORDER (1) *Coccidia* (zygote not motile ; sporozoites enveloped)

Family *EIMERIIDÆ* (Gametocytes similar ; microgametocyte develops into many microgametes)

Genera : *Eimeria*, *Isospora*, *Cryptosporidium*, *Tyzzeria*

Family HÆMOGREGARINIDÆ (with two hosts ; in the circulation of vertebrates and in the gut of invertebrates)

Genus : Hepatozoon

ORDER (2) *Hæmosporidia* (zygote motile ; sporozoites naked)

Family PLASMODIIDÆ (with pigment granules. Schizogony in the peripheral blood of vertebrates)

Genus : Plasmodium

Family HÆMOPROTEIDÆ (Gametocytes in peripheral blood, schizogony elsewhere)

Genera : Leucocytozoon, Hæmoproteus

Family BABESIIDÆ (no pigment granules. Minute parasites of erythrocytes)

Genera : Babesia, Egyptianella, Theileria

CLASS (4) **THE CILIATA** (having cilia for locomotion)

SUB-CLASS—Euciliata (macronucleus and micronucleus)

ORDER —*Spirotricha* (the adoral zone winds clockwise to the cytostome. The peristome does not extend beyond the general body surface)

Family BURSARIIDÆ—Peristome sunk in a funnel-like hollow at the anterior end.

Genus : Balantidium

PARASITES OF DOUBTFUL POSITION

Parasites of the genera *Anaplasma*, *Eperythrozoon*, *Toxoplasma*, *Haemobartonella*, *Grahamella*, *Sarcocystis* and *Globidium* are not easy to fit into the classification which is indicated above.

CHAPTER II

THE MASTIGOPHORA

GENERAL CHARACTERS OF THE CLASS : THE FAMILY TRYPANOSOMIDÆ : THE GENUS LEISHMANIA

THE protozoa which are included in the class *Mastigophora* are characterised by the presence of flagella which occur in the active fully-grown stages of the organism. They have simple or multiple nuclei and granular *blepharoplasts* from which the *axonemes* (the supporting filaments) of the flagella arise. The *blepharoplast* may be a simple granule at the base of the flagellum. Sometimes several are associated together. In association with the *blepharoplast* there may be a deeply staining granule known as a *kinetoplast*. The term *kinetoplast* is employed by Wenyon (1926) to describe the compound structure consisting of the parabasal body united with a blepharoplast. In *Trichomonas* and allied forms the *axostyle*, which is a stiff rod which commences in the blepharoplast and passes through the centre of the body to protrude with a sharp point at the posterior end, is possibly a modified axoneme. Reproduction in the Mastigophora is usually by binary fission, the longitudinal division commencing, as a rule, at the flagellated end of the organism after the blepharoplast and nucleus have divided.

Most of the class are free-swimming organisms which live in a fluid environment which may be fresh or salt water or, for example, the gut contents of domestic animals.

Encystation, as a means of protection and dispersal, commonly occurs within the *Mastigophora* but not with many of those of veterinary importance.

CLASSIFICATION

There are two sub-classes, the *Phytomastigina* which includes those *Mastigophora* which resemble plants owing to the fact that they are provided with chromatophores which contain chlorophyll and the *Zoomastigina* which do not contain chlorophyll and which in this sense are more akin to higher animals.

The sub-class *Zoomastigina* is divided into four Orders, of which three contain species of veterinary importance—the *Protomonadina*, characterised by the possession of one or two flagella and including the important Family the *Trypanosomidæ*; the *Rhizomastigina* in which locomotion is by pseudopodia as well as by flagella (including the Family *Mastigamœbidæ*) and the *Polymastigina* (with three to eight flagella) which contains the Families *Trichomonidæ* and *Hexamitidæ*.

The important genera in these families will be described. It must be appreciated, however, that in addition there are very many members of the *Mastigophora* which are coprozoic and very many which appear to exist in domestic animals as relatively benign parasites. Some knowledge of these may be required in order to avoid confusion with the pathogenic forms. A few of the more important genera are figured on page 73 but for further information the reader is referred to the standard text-books by Wenyon (1926) or Kudo (1954).

The Family *TRYPANOSOMIDÆ* Doflein, 1901

Members of this Family are characteristically leaf-like. Typically all have a single flagellum, which arises from a blepharoplast. They are parasites in vertebrates, invertebrates or plants. In some, part of the flagellum forms the outer margin of an undulating membrane which extends along one side of the body.

GENERAL MORPHOLOGY

During the course of their development in vertebrate and invertebrate hosts, members of the Family may pass through a number of characteristic developmental stages which are distinguished morphologically.

(1) *The trypanosome stage*

In general shape, trypanosomes resemble a curved flattened blade with tapering ends. The vesicular nucleus is usually situated about the centre of the body and contains a large karyosome. A kinetoplast is situated near the posterior end and from it an axoneme runs forward to the anterior end of the body passing

along the border of the undulating membrane which arises from the convex edge of the body as a thin ridge of cytoplasm. At the posterior end the membrane terminates. The axoneme may or may not be continued into a flagellum. In *Trypanosoma congolense* and in the stumpy form of *T. brucei* the axoneme terminates at the anterior extremity. In most other forms, however, it extends anteriorly as a free flagellum.

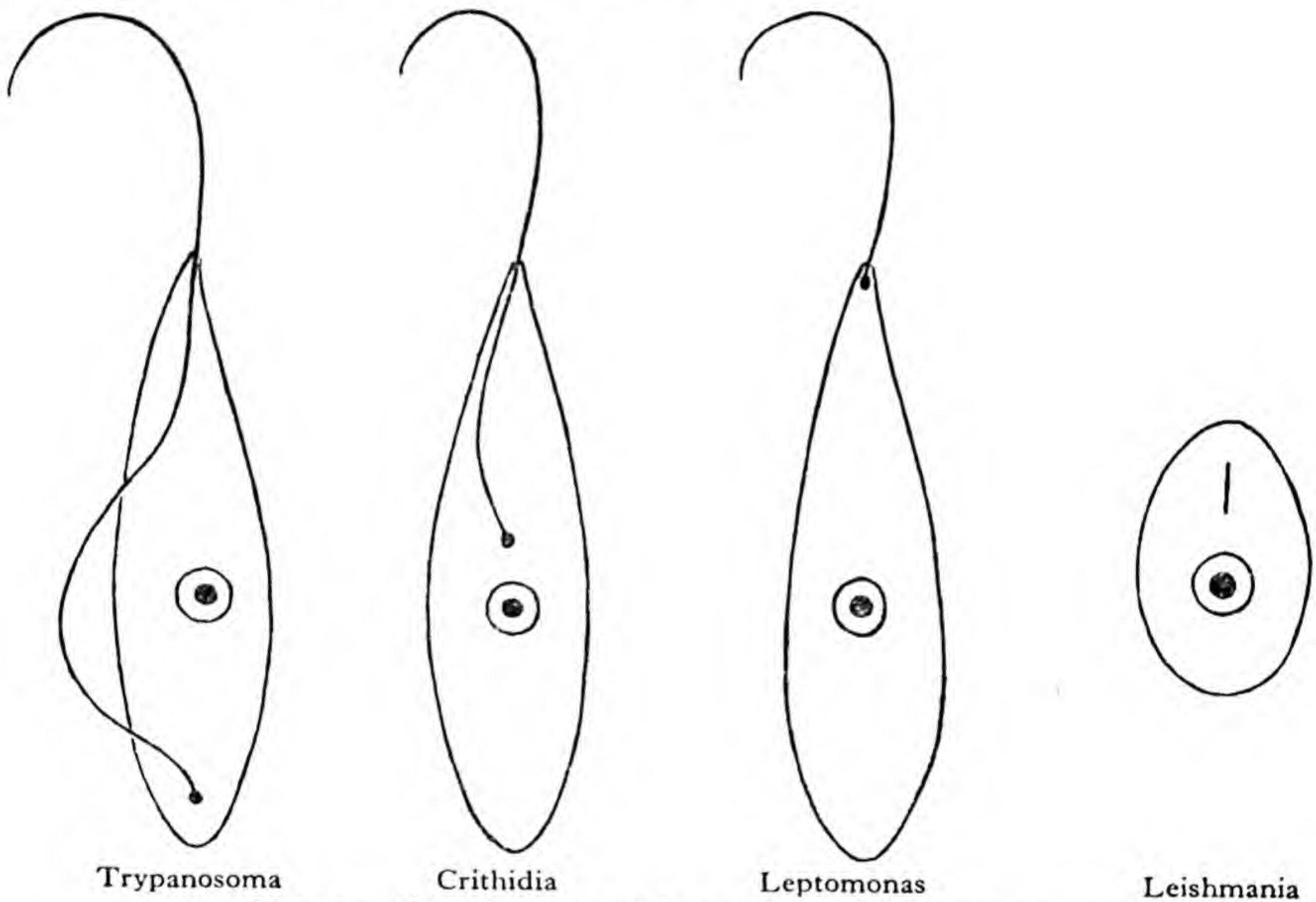


FIG. 1.—Developmental forms of trypanosomes.

(2) *The crithidial stage*

This stage has an elongate body ; the kinetoplast and the origin of the flagellum are situated in the middle of the body, near the nucleus.

(3) *The leptomonas stage*

This stage also has an elongated body but the kinetoplast and the origin of the flagellum are situated in the anterior part of the body in front of the nucleus.

(4) *The leishmanial stage*

This comprises a rounded body ; the flagellum is absent but the kinetoplast is present.

DIVISION INTO GENERA

The Family is divided into genera according to the degree of development reached.

(1) *Leishmania*—these do not develop beyond the leishmanial stage in the vertebrate host, though they develop to the leptomonal stage in the invertebrate host or in culture.

(2) *Leptomonas*. This genus is confined to invertebrates and does not develop beyond the leptomonal stage.

(3) *Phytomonas*. Resembles *Leptomonas* but all species occur in plants and are transmitted by invertebrate intermediate hosts.

(4) *Crithidia*. This genus shows leptomonal and crithidial stages during development. The genus is confined to invertebrates.

(5) *Herpetomonas*. Develops to the trypanosomal stage but is confined to invertebrates.

(6) *Trigomonas*. Develops to the trypanosomal stage in plants.

(7) *Trypanosoma*. Develops to the trypanosomal stage in vertebrates.

Genus *LEISHMANIA* Ross, 1903

In the course of their life-cycle in mammalian and insect hosts members of the genus pass through two stages of development—the rounded leishmanial form and the flagellate leptomonal form. In the mammalian host the parasite occurs only as a leishmanial stage. Leptomonads are seen in the insect vector and in culture.

Morphology. The organisms are circular or oval, about $4.0\ \mu$ by $2.0\ \mu$ and are surrounded by a definite membrane. The nucleus is spherical and often seen to one side of the parasite. The kinetoplast is rod shaped. In well stained specimens, a faint line representing the axoneme can be seen running through the cytoplasm. The organisms stain well with Romanowsky stains

FIG. 2.

LEISHMANIA and TRYPANOSOMA

a, b, c. Leishmania in tissue smears.

a. Macrophage packed with parasites.

b. Parasites scattered by rupture of host-cells.

c. Detached portion of host-cell with parasites.

d. Erythrocytes.

e-t. Trypanosomes.

e-h. *T. brucei*.

e. Slender ; *f.* Intermediate ; *g.* Stumpy.

h. Posterior-nuclear forms.

e, f. *T. evansi* and/or *T. equiperdum* ; *i.* *T. vivax* ; *k.* *T. uniforme* ;
l, p. *T. congolense* ; *l, m, n, o.* *T. simice* ; *q.* *T. theileri* ; *r, s.* *T. cruzi* ;
t. *T. lewisi*.

N.B.—This has been modified a little from the original in Hoare (1949).

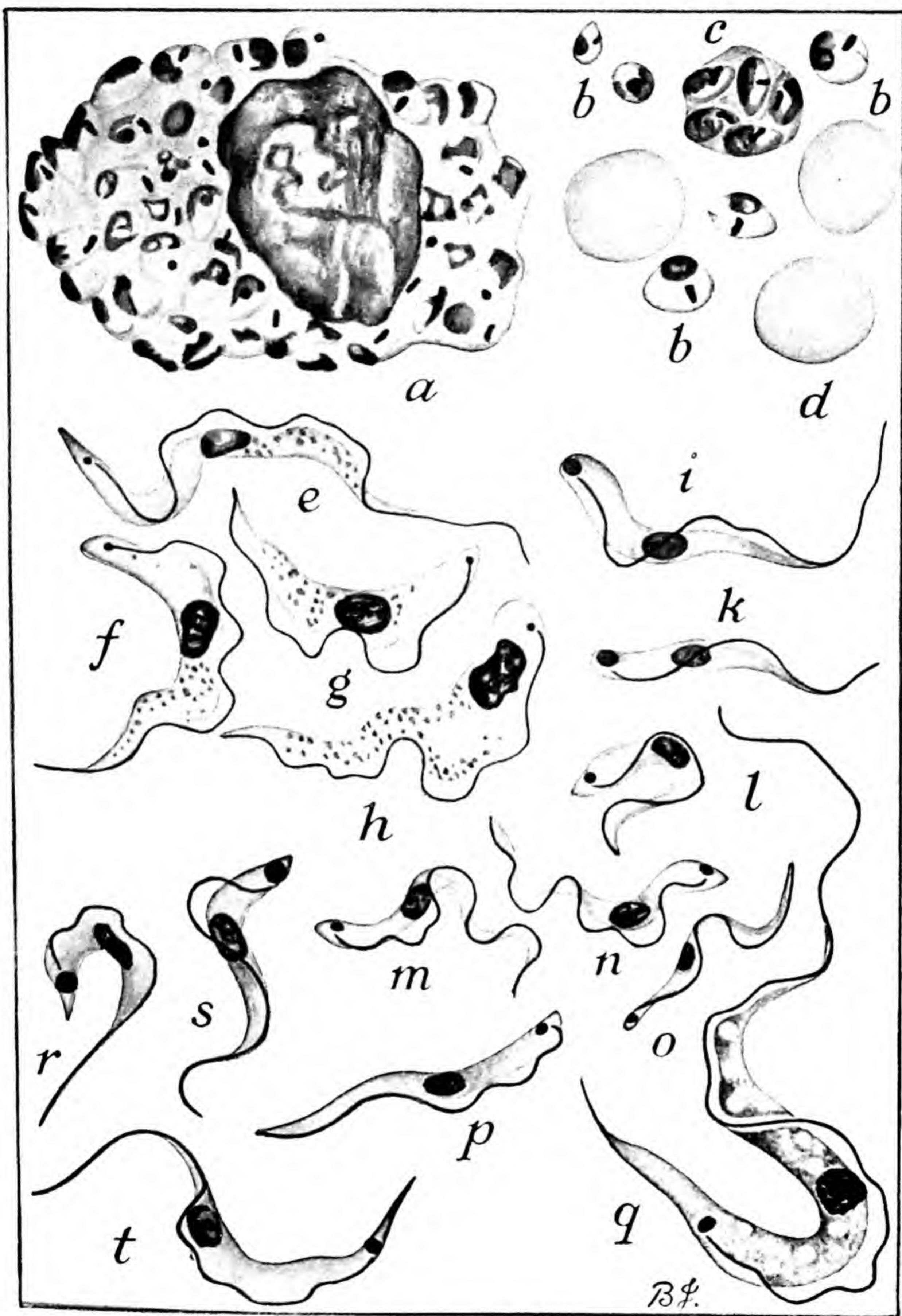


FIG. 2.

and are usually seen in clusters in the cytoplasm of macrophages and endothelial cells. In stained tissue smears the parasites may appear outside the host cells but it can be assumed that this has occurred through rupture of the cells during fixing and staining.

Distribution. The genus is parasitic in man, dog, and some other mammals including the Chinese and golden hamsters and the cotton-rat. Hamsters and cotton-rats are very suitable laboratory hosts. Sand-flies (blood-sucking insects of the genus *Phlebotomus*) act as the insect vectors.

Four forms of human leishmaniasis have been described :—

(1) A visceral form occurring in India and Eastern Asia, mainly affecting the spleen and attacking adults, known as Kala-Azar, the causal organism of which has been called *Leishmania donovani* (Laveran and Mesnil, 1903).

(2) A visceral form occurring in the Mediterranean littoral, mainly attacking children and called *L. infantum* (Nicolle, 1908).

(3) A cutaneous form occurring in the Middle and Far East, mainly attacking adults, the organism being called *L. tropica* (Wright, 1903).

(4) A form which may be either cutaneous or visceral, occurring in Brazil, the organism being *L. brasiliensis* Vianna, 1911.

The classification of these leishmanias still remains in doubt largely because the parasites are morphologically indistinguishable. Hoare (1949) recognises no more than two valid species :—*L. donovani* (synonyms *L. infantum*, *L. chagasi* and *L. canis*), and *L. tropica* (synonyms *L. brasiliensis*, and *L. tropica var canis*). Many attempts have been made to distinguish different species by biological methods, e.g. their growth in culture, by fermentation reactions, and by the serological methods. The experimental differentiation of the parasites is considered by Kirk (1950). In the dog, cutaneous and visceral forms of the disease exist in the same areas. It is possible that there is only one disease entity, the cutaneous lesions being late manifestations of the condition.

It must be remembered that all the human forms are transmissible to the dog by inoculation and set up the typical canine disease. Since the 1939-45 war there has been considerable evidence of an increase in distribution of the disease presumably as the result of movements of population, both on the continent of Europe and in the Far East.

CANINE LEISHMANIASIS

The canine disease is prevalent round the shores of the Mediterranean, in Asia Minor and Arabia and in many other countries. *Leishmania donovani* appears to be a natural pathogen of dogs. The visceral form of the disease as found in children in the Mediterranean area is closely related epidemiologically to that found in dogs. Systematic destruction of dogs has been shown markedly to reduce the incidence of disease in children. By contrast, the Indian and Sudanese forms of human leishmaniasis appear to exist independently of dogs as a source of infection. Dogs are also found infected with *L. tropica* both in the Old and in the New World, the disease being manifested by skin lesions of the same type as those found in man.

PATHOGENICITY AND PATHOLOGY

The disease in dogs is characterised by anæmia, emaciation and general debility. When the skin becomes affected, ulceration may develop particularly at the angle of the lip and on the eye-lids. In more chronic cases, a generalised furfuraceous eczema with loss of hair over a wide area may occur. In advanced cases, diarrhœa appears as a terminal symptom. It is not known whether dogs can make a spontaneous recovery from infection. Chronic cases with characteristic symptoms usually seem to terminate in death but there appear to be many instances in which the only symptom is a persistent eczema. The apparent absence of the canine disease in areas where human leishmaniasis is common may often be due to failures in diagnosis.

Lesions. Anæmia and emaciation, enlargement of the liver and spleen, congestion of the bone marrow, cloudy swelling of the kidneys and sometimes ulceration of the stomach in addition to ulceration of the skin.

Diagnosis. The only really reliable means of diagnosis is by demonstration of the parasite which is found principally in the peripheral blood, spleen, lymph glands, bone marrow, liver and skin. In the dead animal stained smears from the spleen and bone marrow are the most reliable. Positive smears are usually obtained from the tibial bone marrow. Gland puncture is reliable in early cases. Bone trephining or spleen puncture may not be considered

advisable in the living dog but skin scrapings, particularly those taken from the edges of ulcers, frequently contain organisms. In some instances blood culture has revealed infections which direct examination has failed to show.

None of the serological tests is entirely reliable, but the formol-gel test is probably more reliable in the dog than in man. The basis of this test is the addition of a drop of commercial formalin to one ml. of the serum of the suspected animal. In a positive case the serum solidifies in a few minutes, very quickly becoming opaque like the coagulated white of an egg. The reaction is not, however, very specific. The diagnosis of leishmaniasis is fully considered by Shortt (1947).

CONTROL

(a) **By control of the dog population**

Transmission is often through the medium of diseased and neglected dogs which should accordingly be destroyed in any area where the incidence of *Leishmania* is high. It must be remembered that there is an important public health aspect.

(b) **By control of sand-flies**

D.D.T. sprayed on the walls of houses and in other appropriate places as a 5 per cent. solution has been shown to be reasonably effective in the control of *Phlebotomus*.

(c) **By therapy**

Considerable investigation into the problems of therapy, particularly, of course, with reference to the human disease, has shown that *Leishmania* can be controlled by the use of such antimony compounds as neostibosan and sodium stibogluconate, and by stilbamidine and other aromatic diamidines. The Mediterranean type of the disease differs from kala-azar in that it does not respond so readily to neostibosan but antimony gluconate (solustibosan) is reported to be effective. Adler has reported failures to cure canine leishmaniasis using stilbamidine. Even after intensive treatment a residual infection remains. It is probable that relapses occur after apparent recovery following treatment with any of the known drugs. With the antimonials, as with the arsenicals, the pentavalent compound must apparently be reduced in the body to the trivalent form before it is effective.

In human medicine it has been suggested that a full, balanced diet, with adequate vitamin intake is highly important during treatment.

IMMUNITY

There is some evidence of both natural and acquired immunity to kala-azar. It is possible that the high incidence of leishmaniasis in the Mediterranean area among children, as compared with adults, is the result of children, living in close contact with infected dogs, commonly becoming infected and thereby attaining resistance as adults. In India, where there does not appear to be a reserve of infection among animals, adults commonly remain susceptible, there being a lower incidence among young children.

As already indicated, it seems probable that a proportion of dogs carry infection with only mild clinical symptoms. It seems improbable, however, that a sterile immunity ever results. With *Leishmania tropica* in man, a single infection terminating in spontaneous recovery, appears to result in a solid resistance to reinfection. In countries where the cutaneous form of leishmaniasis exists it is said to be the custom to inoculate children on an inconspicuous place in order to prevent the later development of disfiguring ulcers or scars on the face. There is, however, experimental evidence that full resistance develops only if the disease has run its full course. In the presence of an early lesion, reinoculation with normal sequelæ can occur. In later stages of the disease, however, the development of the second infection is retarded.

TRANSMISSION

The vectors of *Leishmania* are sand-flies, which are Dipterous insects belonging to the genus *Phlebotomus*. When the sand-fly feeds on the mammalian host the parasites are ingested in the wandering macrophages or monocytes which harbour them. Sometimes the infection may be acquired from the skin lesions. In the mid-gut of the insect the parasites change from the leishmanial to the leptomonial stage of development. These multiply very rapidly. The mid-gut of different species of sand-fly seems to show a specific selectivity for particular strains of *Leishmania* and there is considerable variation in behaviour according to the

strain of the parasite and the species of the fly. In many instances there are enormous numbers of parasites in the mid-gut by the third day after infection. The flagellates spread forwards and migrate to the œsophagus and pharynx which they eventually block. When the sand-fly feeds on a fresh host the plug of flagellates interferes with the flow of blood but is eventually discharged and sets up a fresh infection. There is no evidence of a special infective stage of the leptomonad.

After entering the mammalian host the parasites develop again into the leishmanial form and are engulfed by macrophages of the subcutaneous tissues. From this site they may either spread by local extension, in the cutaneous form of the disease, or be carried about the body in the more generalised form of disease. Adler (1947) fed laboratory-bred sand-flies on the flagellate stages in culture and on leishmanial stages in heavily infected hamster spleen and has described in detail the development in the sand-fly.

CHAPTER III

THE GENUS TRYPANOSOMA Gruby, 1843

INCIDENCE OF INFECTION : DEVELOPMENT AND TRANSMISSION OF THE PARASITES

FLAGELLATES of this genus attain the *trypanosome* structure at some stage of their development. All occur as parasites in the blood and tissues of vertebrates. Like the other members of their Family they are flattened in shape. Usually they are pointed at the flagellate (anterior) end and bluntly rounded or pointed at the other. The nucleus is central. There is a blepharoplast from which the flagellum arises and runs towards the opposite end, marking the boundary of the undulating membrane. Usually the flagellum extends freely beyond the body. Multiplication is by binary or by multiple fission. The organism is usually transmitted by an insect vector in the alimentary tract of which it undergoes a series of developmental stages.

The genus is of outstanding importance because among the species it includes those which cause the disease of *trypanosomiasis* in man and in his domestic animals.

GEOGRAPHICAL DISTRIBUTION AND INCIDENCE OF INFECTION

The trypanosomes which cause disease in domestic animals occur principally in the African Continent, their main distribution following that of the tsetse flies (species of *Glossina*) which are the main vectors between 15° N. and 15° S. of the equator. There are, however, several species which do not require tsetse flies for transmission. *T. evansi* which causes *surra* in horses and camels and a similar disease in dogs is found both in Africa and in Asia outside the range of the tsetse flies. In South America *T. equinum* causes the disease of horses known as Mal de Caderas. *Dourine*, a venereal disease of horses and other equines caused by *T. equiperdum* formerly had a wide range throughout the world but as a result of vigorous measures taken for its eradication it is now much more limited, being confined to limited areas in Africa, Asia, South America and Europe. *T. vivax*, although

typically transmitted by tsetse has nevertheless managed to establish itself in Central and South America and in the West Indies in the absence of *Glossina*. Most domestic animals are in some degree susceptible to infection with trypanosomes. Cattle are excluded from large areas in tropical Africa owing to their susceptibility to infection with the dominant species *T. congolense* and *T. vivax*, which are the principal parasites involved in the disease known in southern Africa as *nagana*. The actual incidence of infection varies according to such factors as the density of the *Glossina* population and the system of husbandry adopted locally, but with rare exceptions cattle and trypanosomes cannot coexist in Africa and the enormous areas in which stock, and consequently man, cannot live are far more suggestive of the importance of the disease than are the figures which indicate the incidence of infection in those areas where stock can maintain a more or less precarious footing.

Throughout the world there are, in addition, species of trypanosome which are not pathogenic to their hosts and both cattle and sheep in temperate climates may carry a usually light infection. *T. theileri* is a large trypanosome found throughout the world in cattle while *T. melophagium* occurs commonly in sheep in Britain and many other countries. These two species are very rarely pathogenic.

LOCATION OF THE PARASITE

Trypanosomes are essentially parasites of the blood plasma and other tissue fluids of vertebrates and of the alimentary tracts of insects. Recently Fiennes (1952), following Schwetz (1928), has called attention to the occurrence of trypanosomes in particular organs, notably the heart and some of the endocrine glands. The distribution of the parasite within the body appears to vary with the species. As described by Hornby (1949) *T. congolense* and *T. simiae* are essentially blood parasites and will nearly always be found in blood smears but other African trypanosomes are found only incidentally in the peripheral blood. *T. brucei* appears to invade all the body organs.

Those trypanosomes, which are of veterinary interest, are capable of parasitising a wide range of mammalian hosts.

T. congolense, for example, has been reported to cause disease in every kind of domestic animal, although the pig may show resistance. In addition, very many species of wild game in Africa appear to be able to harbour trypanosomes without disease resulting.

These species of trypanosome can complete their full developmental cycle only in species of a single genus of insects—*Glossina*. Transmission of the disease by other insects is purely “mechanical,” the parasite does not go through any developmental stages and the infectivity of the mechanical vector is of very limited duration.

LIFE-CYCLE AND REPRODUCTION OF THE PARASITES

The life-cycle involves an alternation between the vertebrate and the invertebrate host. Mammalian blood containing parasites is taken into the intestine of the insect vector. Usually the majority of the parasites disappear but after about a week multiplication of the remaining parasites occurs and long slender trypanosome forms develop. From this stage one of two courses may be followed. In one type of development the trypanosomes are carried backwards to the rectum where they assume a crithidial form (see above) but finally develop again into trypanosome forms which are passed out with the fæces of the insect onto the skin of the vertebrate host. This type of development, which is called “development in the *posterior station*” is not characteristic of tsetse flies. The second type of development occurs in tsetse flies. The trypanosomes migrate forwards from the intestine and invade the proboscis and in some instances the salivary glands of the fly and crithidial forms and subsequently small trypanosomes (metacyclic forms) develop in these *anterior stations*. In this type of development the parasites are injected into the definitive host by the bite of the insect.

Development in the posterior station

Trypanosoma lewisi of the rat is an example of a trypanosome developing in the posterior station. After ingestion, the trypanosomes enter the cells lining the stomach of the rat flea and form pear-shaped bodies which grow in size whilst the kinetoplast

and nucleus multiply by repeated division. Axonemes develop from the daughter kinetoplasts and form new flagella. The bodies divide into a number of trypanosomes which resemble the blood forms. The cell ruptures and the trypanosomes escape into the stomach of the flea. The next stage is the migration backwards of trypanosomes which, as they leave the stomach, evince a loss of activity, shortening of the body, rounding of the posterior end and a displacement of the kinetoplast until it is anterior to the nucleus, thus giving the crithidial structure.

Multiplication of these crithidial forms occurs with the formation of small trypanosome forms which are passed out with the fæces onto the skin of the rat, at the time when the flea bites. This is the infective stage and organisms may invade the rat by contamination of the bite wound with flea fæces or they may be licked up by the rat when trying to allay the irritation of the bite. They then invade the animal by penetration of the mucous membrane of the digestive tract.

Development in the anterior station

In the case of *Trypanosoma vivax* most trypanosomes entering the gut of the tsetse fly quickly degenerate but some organisms are detained in the proboscis and these develop in the labial cavity into crithidial forms with free flagella. The parasites then pass into the hypopharynx where the infective metacyclic trypanosomes develop. These are small trypanosomes which resemble the forms which occur in vertebrate blood. All the stages have free flagella.

T. brucei is ingested with the blood taken by the tsetse fly and development commences in the mid-gut where long slender forms are produced. These migrate backwards but when they reach the end of the peritrophic membrane (the chitinous dialyser lying in mid- and hind-gut) they enter the space around it and penetrate into the proventriculus from which they migrate to the hypopharynx and salivary glands. Here the crithidial and metacyclic forms are produced. All the developmental forms in the tsetse fly have free flagella except the metacyclic forms which resemble the stumpy forms of the blood. The developmental cycle of a trypanosome such as *T. brucei* in *Glossina morsitans* takes 25 days or more, and up till the end of that time the fly is not infective.

T. congolense has a similar cycle of development in the tsetse fly to *T. brucei*—some development occurring in the mid-gut. Parasites have been detected in the ectoperitrophic space but they do not invade the salivary gland. No free flagella appear, however, at any stage.

Development in the mammal

No sexual differentiation has been detected in trypanosomes and no syngamy appears to occur in the life cycle. Robertson (1912) claimed that some specialisation of blood forms occurred and that in the case of *T. gambiense* of man it was the stumpy forms which persisted and initiated the development in the invertebrate ; the long forms being destroyed in the intestine of the fly.

Multiplication in the vertebrate occurs by binary fission, division commencing at the kinetoplast. This is followed by division of the nucleus and cytoplasm. The flagellum remains attached to one of the daughter kinetoplasts, another flagellum growing from the other. Division is not always equal.

It has been shown experimentally (Nash, 1957) that metacyclic forms of *T. rhodesiense* develop and multiply within the subcutaneous tissues, this being related to the appearance of a local reaction with a characteristic carbuncle-like swelling.

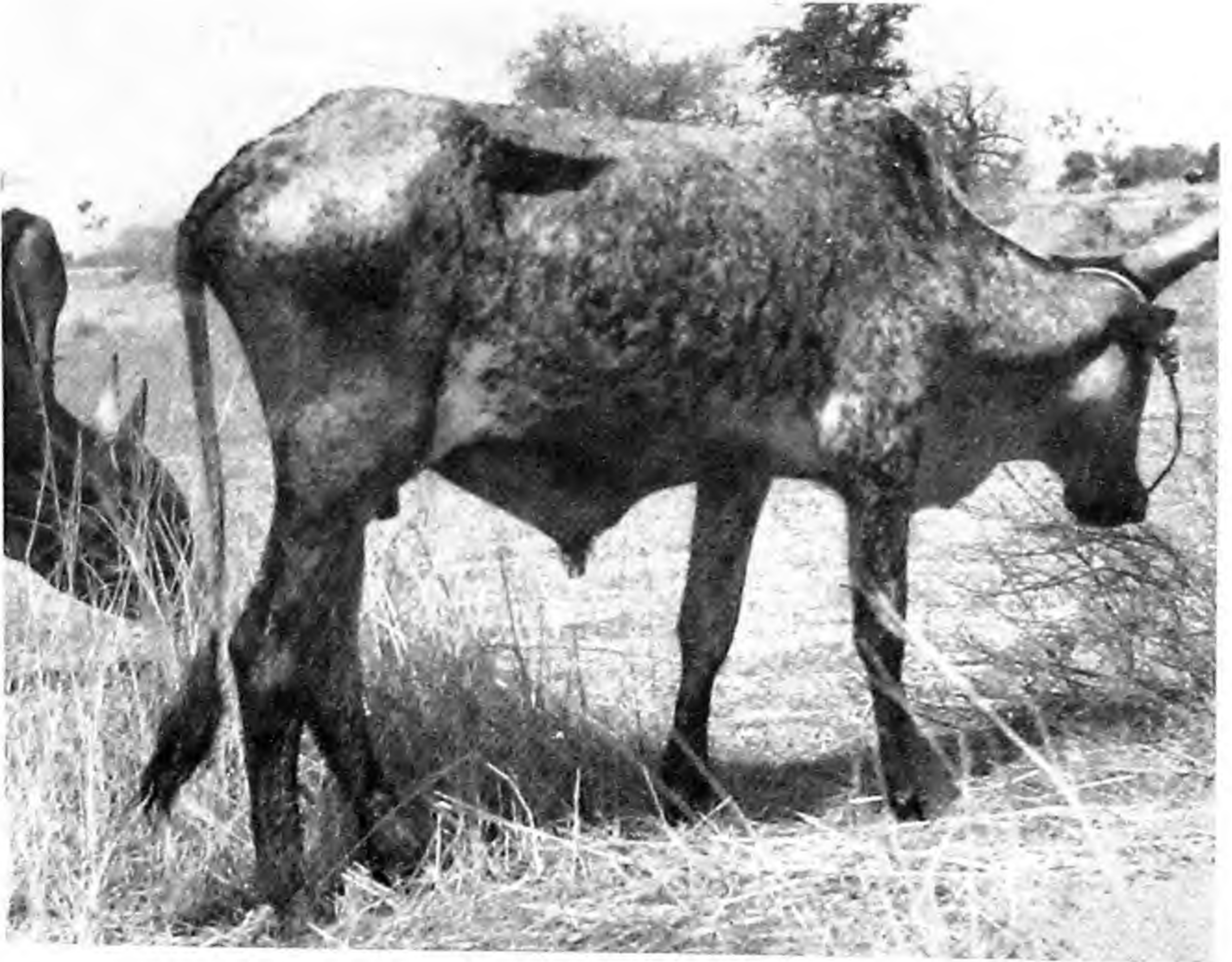
In some instances (notably *T. cruzi*) the organisms invade the tissue cells of the definitive host and assume a leishmanial form. Large numbers of these forms may occur in a single cell and these aggregates have been interpreted as schizonts. There is, however, no real evidence that schizogony occurs. It has been claimed that all trypanosomes invade cells to some extent and it has been suggested that the periodic disappearance of the parasites from the peripheral blood may be due in part to a developmental cycle in the vertebrate host and not entirely to the destructive action of the host's defences.

During development, either in the vertebrate or in the invertebrate, crithidial, leptomonial and leishmanial forms may appear. In a single blood film from an infected vertebrate it will often be noted that not all of the parasites are of the same shape and size. Different forms of the same species with marked morphological variation are characteristic of the group of trypanosomes

FIG. 3



Typical habitat of *G. morsitans* (Northern Nigeria)



Bull suffering from trypanosomiasis (Northern Nigeria)
Photos : W. Petana

FIG. 3



Glossina morsitans feeding and at rest. Photo : W. Petana, West African Institute for Trypanosomiasis Research

known as *polymorphic*. Changes in morphology have been related to changes in numbers which have in turn been related to the antigenic lability of the parasite. Thus, there is good evidence that the rising numbers of parasites in a relapse is controlled and reduced by developing antibodies and that the next relapse, following the remission so caused, is due to an antigenically different trypanosome. In experiments recorded by Ashcroft (1957) it was shown that the proportion of stumpy forms of *T. brucei* and *T. rhodesiense* increased and the proportion of slender forms decreased as the relapse progressed. It seemed probable that antibodies were responsible for the appearance of stumpy forms. If a considerable degree of uniformity exists among the parasites found in vertebrate blood the trypanosome is known as *monomorphic*.

TRANSMISSION OF TRYPANOSOMES

(a) By coitus

T. equiperdum is unique among the trypanosomes in being transmitted without the intervention of an insect vector. It is essentially a venereal disease and the parasites pass from one animal to another during the sexual act.

(b) By biting flies

T. evansi, *T. equinum* and, in some parts of the world, *T. vivax* are habitually transmitted by biting flies, particularly by species of *Tabanus* and *Stomoxys*. All the African trypanosomes ordinarily transmitted by tsetse may sometimes be transmitted in such a way. Because transmission by biting flies is mechanical and does not involve any cycle of development within the fly, the fly is infective immediately after feeding but remains so only for a short time. Mechanical transmission of trypanosomes occurs when the fly is interrupted during feeding. It is necessary for the fly to complete its meal on a second host without delay which would lead to the death of the trypanosome. Successful mechanical transmission probably requires a locally high density of the host. It must be remembered that tsetse flies themselves may under suitable conditions carry out mechanical infection. Buxton

(1948) expresses the view that transmission of the cattle trypanosomes in Africa by biting flies other than *Glossina* is common and serious.

(c) **By tsetse flies**

The factors which govern the transmission of the African trypanosomes are not entirely understood. If a batch of tsetse flies is fed on an infected vertebrate not all the flies become infected. With experimental flies the rate of infection is usually about 10 per cent. and it rarely exceeds 20 per cent. The percentage rate of infectivity may, however, be increased considerably by exposing flies or the fly pupæ to unusually high temperatures (Lloyd, 1930). With the polymorphic trypanosomes the rarity of cyclical infection in wild caught flies is in fact quite remarkable and it usually does not exceed about one in a thousand.

It is thought that the state of development of the trypanosome at the time of feeding may influence the rate of infection and that, for instance, no infection with *T. brucei* can occur if no stumpy forms are present in the blood. Duke (1935) says that strains of trypanosomes which have been mechanically transmitted lose their power of developing in tsetse and that prolonged residence in one host will also affect the transmissibility. It seems probable that this phenomenon is connected with a change in the developmental cycle of the trypanosome in the vertebrate.

It seems also that the species of the vertebrate host may affect the transmissibility and Corson (1935) got an experimental rate of 60 per cent. in *G. morsitans* fed on a reed-buck infected with *T. rhodesiense*. Duke found calves to be unsuitable hosts in that residence in them reduced transmissibility. There also seems to be a selective relationship between certain species of trypanosome and certain species of tsetse fly. Thus *G. morsitans* and *G. swynnertoni* seem to be very much more efficient transmitters of *T. rhodesiense* than is *G. pallidipes*, which on the other hand seems to be a very efficient transmitter of *T. brucei*. *G. palpalis* appears to be inefficient as a transmitter of *T. congolense* and cattle often thrive in areas infested with this tsetse fly. There is no doubt that the trypanosome can survive in this fly but strains of the organism appear to die out in *G. palpalis* areas unless there is an efficient alternative vector.

(d) Other methods of transmission

Intra-uterine transmission has been indicated with several species of *Trypanosoma* (notably *T. equinum*) while *T. evansi* has been transmitted in the milk to a sucking puppy. There are several recorded instances of animals becoming infected after eating the organs of other animals.

It may not be out of place to mention the possibility of needle passage during the course of mass inoculation campaigns against other diseases. Trypanosomes are readily transmitted by inoculation of infected blood.

CHAPTER IV TRYPANOSOMIASIS

THE SPECIES CONCERNED : PATHOGENICITY : IMMUNITY :
DETECTION OF INFECTION

SPECIES DIFFERENTIATION OF THE TRYPANOSOMES

THE species are differentiated by their morphological characters, particularly when in the blood of the vertebrate, by the stages and course of their development when in the insect host and on biological features such as mode of transmission, pathogenicity and host specificity. Morphological characters by which the species may be differentiated include the size and shape of the body, variations in the size and position of the nucleus and kinetoplast and degree of development of the undulating membrane and flagellum.

It must be remembered, however, that there may be considerable morphological differences between individual parasites of the same species, particularly according to their state of development. As already indicated, trypanosomes may pass through the crithidial, leptomonal and leishmanial forms during part of their developmental cycle. With the polymorphic trypanosomes markedly different forms appear in the vertebrate blood.

There is a very long list of named trypanosomes in the literature, very many of the names being synonyms. According to Hornby (1949) the following species are those of veterinary interest :—

<i>T. theileri</i>	Laveran 1902
<i>T. brucei</i>	Plimmer and Bradford 1899
<i>T. gambiense</i>	Dutton 1902
<i>T. rhodesiense</i>	Stephens and Fantham 1910
<i>T. congolense</i>	Broden 1904
<i>T. simiae</i>	Bruce <i>et al.</i> 1911
<i>T. vivax</i>	Ziemann 1905
<i>T. uniforme</i>	Bruce <i>et al.</i> 1911
<i>T. evansi</i>	(Steel 1885)
<i>T. equinum</i>	Voges 1901
<i>T. equiperdum</i>	Doflein 1901
<i>T. cruzi</i>	Chagas 1909.

The trypanosomes of medical and veterinary importance are well arranged in a key by Hoare (1949). In general they may be grouped as follows :—

(1) **The Lewisi group**

including **T. theileri**, **T. melophagium**, **T. lewisi** and **T. cruzi** : These trypanosomes develop in the posterior station of the invertebrate. Morphologically, organisms of this group have a tapering posterior extremity; the large kinetoplast is placed at some distance from the posterior extremity and may be nearer the nucleus than it is to the posterior end. The undulating membrane is poorly developed and the anterior part of the flagellum is free.

(2) **The Vivax group**

including **T. vivax** and **T. uniforme** : These trypanosomes normally develop in the proboscis of the tsetse fly. They are characterised morphologically by a bulbous posterior extremity, a terminal kinetoplast, a poorly developed undulating membrane, and a free flagellum. They show great motility, passing rapidly across the field of the microscope.

(3) **The Congolense group**

including **T. congolense** and **T. simiae** : These trypanosomes ordinarily develop in tsetse flies, development commencing in the gut and being completed in the proboscis. Morphologically, the organisms are characterised by their small size, by the absence of a free flagellum and the marginal position of the kinetoplast. The undulating membrane shows moderate development.

(4) **The Brucei group**

includes **T. brucei**, **T. gambiense** and **T. rhodesiense** : Cyclical development in the tsetse fly commences in the gut and is completed in the proboscis and salivary glands. Morphologically, the trypanosomes are characterised by polymorphism. They occur in long, intermediate and short forms. In the long forms there is a long free flagellum, in the intermediate forms there is a short free flagellum while there is no free flagellum in the short forms. The undulating membrane is well developed and the kinetoplast is subterminal. The organisms are sluggish in movement.

(5) The Evansi group

includes *T. evansi*, *T. equinum* and *T. equiperdum*: There is no known cyclical development. Organisms of this group differ from those of the *brucei* group in the absence of polymorphism. Stumpy forms are very rare. Most individuals resemble the long forms of *T. brucei*. Hoare (1957) classed together the *brucei* and *evansi* groups.

Relationships between the species

The exceedingly great similarity between *T. brucei*, *T. gambiense* and *T. rhodesiense* is of outstanding interest. In practice, these three species can be separated only by reference to their pathogenicity. Morphologically they appear to be identical. *T. brucei* can be regarded as non-pathogenic for man but is exceedingly pathogenic for the horse. *T. gambiense* consistently causes a much more chronic type of disease in man than *T. rhodesiense* and it appears to have a different geographical distribution.

T. uniforme appears to bear a very close relationship to *T. vivax* from which it differs only in size.

The *evansi* group is, of course, sharply demarked from the rest of the pathogenic trypanosomes in that the species are never dependent on tsetse flies for transmission. *T. evansi* and *T. equiperdum* are morphologically indistinguishable. *T. equinum* differs from *T. evansi* in the absence of a kinetoplast but strains of *T. evansi* without a kinetoplast do exist and it can be assumed that *T. equinum* originated in this way. *T. equiperdum* may also have originated from *T. evansi* as a strain readily capable of transmission from mammal to mammal during coitus.

PATHOGENICITY AND PATHOLOGY

Variations in pathogenicity

In Africa it is probable that the trypanosomes are essentially non-pathogenic in the wild game which serve as the reservoir hosts. In South America the capybara appears to act as a reservoir for *T. equinum*. In Asia, *T. evansi* is rarely pathogenic in cattle and buffalo.

Among the domestic animals which they parasitise the

trypanosomes show pathogenicity in widely differing degree. Even among different strains of the same species there may be, as shown by Fiennes (1950), remarkable differences in virulence. Even when the resistance factor on the part of the host is not involved, different strains of both *T. congolense* and *T. vivax* differ widely. They may appear as fulminating infections which cause death within 15 days of the first appearance of the parasites in the circulation or as benign parasites which allow natural recovery to occur with little metabolic disturbance to the host. Acquired immunity and premunity are both characteristic of the disease. In general, needle passage tends to reduce the virulence of a particular strain while tsetse passage enhances it. Passage to an alternative host may reduce the virulence of a strain. Serial passage through the same host species tends to produce a strain with more reliable infectivity and with more predictable characters in that host.

The clinical picture

If the disease is not hyperacute, trypanosomiasis is associated with a fairly constant clinical picture, the disease being distinguished by its chronic character, the intense anæmia and emaciation. Characteristically there is a rise in temperature soon after infection, associated with an accumulation of parasites in the blood. These, however, soon become much fewer and thereafter there may be few symptoms except for a progressive loss in weight which becomes more marked in spite of the animal retaining a good appetite. The general picture as shown by Hornby (1949) is not then easily distinguished on clinical grounds from one of undernourishment or from overwork. Œdema is not very characteristic of infection with *T. congolense* although it is more often seen with *T. brucei*. In some instances œdema of the skin and subcutaneous tissues may be marked ; there may be functional nerve disturbances and an ulcerative keratitis. Chronic enlargement of the lymph glands may be observed during life and swelling of the spleen and liver and hyperæmia of the kidney are frequent though not constant post-mortem findings. Except in the case of dourine (*T. equiperdum*) symptoms which can be ascribed to the central nervous system becoming involved are not as marked among domestic animals as in man.

Tissue invasion

Some trypanosomes, such as *T. brucei*, may be markedly invasive. Hornby (1949) has shown how in a susceptible host the parasite injures the walls of the blood capillaries and after passing through them multiplies freely, causing a great deal of damage as it multiplies. Invasion of the bone marrow may lead to a very marked and early anæmia. The muscles of the heart may be attacked, this leading to circulatory disturbance. Invasion of the hæmato-encephalic barrier causes nerve lesions. By comparison, *T. congolense* is usually less acutely pathogenic. It rarely leaves the blood vessels so that its effect is confined to its action on the blood-forming tissues and to the production of capillary embolism. Nevertheless some strains of *T. congolense* may produce acute disease with rapid death. Fiennes (1950) discussed some anomalies in the pathological picture associated with trypanosome infection of cattle. In particular, as he said, it is difficult to explain the progressive advance of the state of disease occurring contemporaneously with an apparent elimination of the parasite. He was later (1952) able to show, however, the presence of both *T. congolense* and *T. vivax* in the hearts of cattle associated with lesions of sufficient magnitude to account, in his opinion, for the majority of the symptoms and the pathological change associated with the disease.

Blood changes

Nevertheless, in general, the most noticeable pathological change is the progressive anæmia; the number of red blood corpuscles and the hæmoglobin content of the blood being reduced to 25 per cent. of the normal value. This anæmia is not caused by destruction of red cells but by a failure in production. As the disease progresses, a hypoglycæmia develops which becomes severe in the terminal stages. This hypoglycæmia is associated with an exhaustion of the glycogen reserves of the body and it is suggested that it is due to a failure of the liver to lay down a glycogen reserve rather than to an abnormal consumption of sugar, although trypanosomes do consume large quantities of glucose.

French (1938), investigating the blood changes occurring in cattle and sheep infected with *T. brucei* and *T. congolense*, found that towards crisis or death there is an increase in the lactic acid

and a decrease in the sugar content of the blood. There is also an increase in globulin, mainly due to a rise in the euglobulin fraction and a decrease in the potassium content of the blood. This last change is ascribed to the decrease in the red blood corpuscles which are rich in potassium. Zwemer and Culbertson (1939) found in experimental animals infected with *T. equiperdum* an increase in the potassium content of the serum. This they ascribed to destruction of the blood cells. An increase in the sodium chloride content of the blood has also been recorded in *T. congolense* infection of cattle.

Changes in the nervous system

In human sleeping sickness caused by trypanosomes, the principal pathological changes occur in the lymphatic glands and central nervous system. The lymphatic glands are enlarged and show hyperplasia of the follicles, a multiplication of macrophages in the sinuses, round cell infiltration with leucocytic elements and plasma cells, and, in the later stages of the disease, disorganisation resulting in the suppression of glandular function.

The changes in the central nervous system consist of a vascular meningitis with intense lymphocytic perivascular infiltration, followed by an encephalo-meningitis. Mott (1906) described the specific nervous lesions as infiltration with plasmocytic elements derived from lymphocytes and he noted the presence of large hyaline, fuchsinophile, vacuolated cells (*morular cells of Mott*) disseminated in enormous numbers throughout the brain parenchyma. A similar brain picture has been described in monkeys and sheep experimentally infected with *T. gambiense*.

An inflammation of the pia mater occurs in human infections and leads to the escape of trypanosomes from the blood into the cerebro-spinal fluid, in which, however, they do not survive for long. There is also an escape of macrophages from the pia mater leading to an increase in the cellular content of the cerebro-spinal fluid. This increase of cells in the fluid has been used as a method of diagnosis in human sleeping sickness, in which trypanosomes themselves are often undetected.

Other lesions

Other lesions include the enlargement of the spleen and lymphatic tissues, congestion of the bone marrow and of the

stomach and small intestine and in some cases gelatinous infiltration of the sub-cutex.

Round cell infiltration occurs round the blood vessels and biliary canals in the liver, and the blood vessels of the kidney and the epithelial cells of the urinary tubules are degenerated. Two explanations have been given for these lesions, one being that they are evidence of intoxication due to an endotoxin set free from the trypanosomes by lysis and the other being that they are due to invasion of the tissues concerned by trypanosomes. Fiennes' (1952) work to which reference has been made above may be consulted in this connection.

The cause of death

Why death results from trypanosomiasis is not always clear. It is known that trypanosomes consume large quantities of glucose and that during infection there is a decrease in blood sugar accompanied by an increase in the concentration of lactic acid in the blood. It has been suggested that this increase in lactic acid causes asphyxia by interfering with the absorption of oxygen by the hæmoglobin, but it seems unlikely that the concentration of acid in the blood is sufficient. A more likely explanation is that the destruction of glucose throws a strain on the liver which results in disfunction and a resulting toxæmia. Even so the actual consumption of sugar by the parasites, unless excessively numerous, seems unlikely to cause serious disturbance. The inhibition of red cell formation which appears to occur may be explained on the basis of the (so far undemonstrated) existence of a specific toxin or a proteolytic ferment which results in the formation of toxins.

Attention may be called to Fiennes' (1952) suggestion that the cardiac lesions which he describes may be the underlying cause of the pathological state associated with the secondary stages of trypanosomiasis in cattle.

RECOVERY AND IMMUNITY

Antibody production

It was shown by Taliaferro in 1924 that with *T. lewisi*, two antibodies were involved in producing in rats a true state of immunity. The first of these he called "ablastin." It inhibited

multiplication of the trypanosome. The second antibody produced lysis of the parasites. Infected rats usually pass through an acute phase of disease after which they are completely resistant to reinfection. Immune serum from a recovered rat will protect a normal rat against infection and an agglutinating effect can be demonstrated *in vitro*.

More recently Chandler (1958) has indicated that ablastin is an antibody directed against metabolic products and that it is the cause of agglutination as well as the inhibition of reproduction of *T. lewisi*. Immune serum from recovered rats loses most of its potency when absorbed with metabolic products.

Soltys (1957) has carried out a series of studies with *T. brucei* and has shown for example that protective antibody in rabbits can be elicited 5-8 days after infection and that it reaches a peak within 28 days. The antigenic pattern of trypanosomes is reviewed by Weitz (1961).

Premunity

The existence of a state of solid resistance to reinfection is not, however, characteristic of the pathogenic trypanosomes which are of veterinary importance and the resistance which is most often found is usually evinced by the state of toleration which is called "premunity." To attain a true understanding of the immunological reactions of such a parasite as *T. congolense* it is necessary (Fiennes, 1950) to realise that it acts as a relapsing parasite. Following initial infection, the parasite acts as a true antigen and stimulates antibody production. This, however, is followed quite rapidly by an alteration in some way of the antigenic structure of the parasite which in turn usually induces a further production of antibody. The successive waves of parasites which alternate with periods of antibody dominance account for the characteristic regular appearance of parasites in the blood, with associated temperature peaks. Eventually, under such conditions, the disease assumes a chronic character. The end point of the syndrome may be the time when the various antigenic combinations of the parasite are exhausted and a true immunity supervenes, or one of the earlier antigenic structures may reappear in the absence of a strong resistance to its particular type and the whole cycle may recommence.

Absence of demonstrable antibody is characteristic of the condition of true immunity. Presence of antibody, but with a strong tendency to clinical relapse, is characteristic of premunition. The maximum period during which trypanosomes may persist in the absence of reinfection is not known. There is one record of a man who developed sleeping sickness 15 years after his return to Europe from Africa, without any history of disability during his stay in Africa or during the 15 years after return. Fiennes (1952) found cryptic foci of infection in the heart muscle of cattle which had had no contact with tsetse fly for more than a year and which had shown a positive blood or gland smear on one occasion only.

Immunisation against Trypanosomes

Fulton and Lourie (1946) stress that it is the antigenic lability of many strains of trypanosome which accounts for the well-known difficulty of successfully immunising men and beasts against trypanosomiasis in the field. A solid immunity against a particular antigenic type of trypanosome is worth very little in the face of the considerable, if not vast, multiplicity of antigenic types in nature but also in view of the fact that each of these types may have the capacity to change in such a way as to enable the trypanosome to thrive in an animal previously immunised.

Several observers have suggested that *T. congolense* is one of the most antigenically labile of all trypanosomes and it is this character which in part enables it to resist sterilisation following treatment. Failure of treatment, after a non-sterilising drug dosage, is due to the resistance acquired by the host being unable to complete the eradication of the infection, because the trypanosomes are able to reappear in a changed antigenic form.

Resistant strains of cattle

In the field, considerable thought has been given to the possibility of breeding strains of cattle resistant to African trypanosomiasis (Chandler, 1952 and 1958; Mulligan, 1951). In West Africa, cattle of the N'Dama breed, Muturu cattle or West African Shorthorn cattle with a considerable proportion of the N'Dama blood, usually carry symptomless infection. This infection, as in other instances of premunition, is liable to break down under adverse conditions and for example, acute infection

can occur with *T. vivax* in Muturu cattle which have been isolated from infection for several generations. Nevertheless it seems certain that the N'Dama and similar cattle have a considerably higher degree of resistance than have other African cattle. There appears to be, however, an inverse correlation between incidence of disease and size of the beast and under present conditions the N'Dama cattle are of little economic worth.

Hornby (1941) claimed that sucking animals of breeds not ordinarily highly resistant to *T. congolense* may acquire an infection which leads to a real immunity in areas where the disease is maintained by mechanical passage, *i.e.* where the number of strains of trypanosome is limited. Desowitz (1959) similarly suggested that there is evidence that adult immunity is influenced by antigenic stimulation as a calf.

DETECTION OF INFECTION

Field detection

Under field conditions, each area is likely to have a characteristic epidemiological picture of infection usually associated with changes in the grazing habits of stock. When dealing under field conditions with large numbers of livestock in which careful individual examination is not always possible, there may be confusion with chronic malnutrition and with heavy worm infestation associated with malnutrition. *T. congolense* and *T. simiae* should usually be readily identified in thick blood smears. A staining technique which enables specific identification in thick blood films to be rapidly effected has been described by MacLennan (1957). *T. brucei*, *T. vivax*, *T. uniforme* and *T. evansi* are often absent from the circulation and repeated gland smears may be necessary before the parasite can be identified. If facilities are available, trypanosomes are often most readily identified in fresh blood or gland preparations, their motility being apparent using the 4 mm. objective.

Subinoculation of suspected blood into laboratory animals may be used for diagnosis particularly in cases where the parasites are very few.

Stura (1948) describes a method for the diagnosis of *T. equinum* in South America in which blood mixed with 20 per cent.

sodium citrate is centrifuged and the supernatant fluid examined for trypanosomes.

Serological tests

Serological tests are only of great importance in the diagnosis of dourine (*T. equiperdum*) and to a limited extent with *T. evansi* in camels. The complement fixation test is satisfactory if the presence of only one species of trypanosome can be inferred, *e.g.* when looking for *T. equiperdum* on the continent of Europe. It should be remembered that dourine antigen will give a positive reaction in camels suffering from *T. evansi*. Evidently, in this instance, there is a group reaction and it appears that serum of an animal infected with *T. evansi* or *T. equiperdum* may fix complement with an antigen made from *T. brucei*, *T. evansi* or *T. equiperdum*. The antigen used in these tests is usually trypanosome material obtained from the blood of laboratory animals by centrifugation. In the case of *T. cruzi*, antigen has been prepared from material grown on agar plates.

Other serological tests have not been very satisfactory with the exception of the mercuric chloride test for the presence of surra (*T. evansi*) in camels and horses as described by Bennett (1933).

In a recent review, Weitz (1961) considers that our knowledge of the antigens of trypanosomes is not yet sufficient to apply correctly serological methods of diagnosis.



FIG. 4.—*Trypanosoma vivax*. Photo : W. Petana

CHAPTER V

THE TRYPANOSOMES

THE VIVAX GROUP

T. VIVAX Ziemann, 1905 (Synonyms *T. cazalboui* and *T. capræ*)

This is a large monomorphic trypanosome of distinctive appearance. The bulk of the cytoplasm lies posterior to the nucleus giving to this part of the body a swollen, broad appearance. The kinetoplast is terminal. Individuals vary in length from $20\ \mu$ to $26\ \mu$ averaging $22.5\ \mu$ by $3\ \mu$ broad, the free flagellum varying from $3\ \mu$ to $6\ \mu$. There is no stomach phase of development in the tsetse fly and laboratory animals are not readily susceptible to infection. In fresh blood preparations the organism shows great motility.

Distribution

This trypanosome is widely distributed throughout Africa where it is transmitted by a number of tsetse flies. The parasite has, however, managed to establish itself in several countries (Mauritius, the West Indies and parts of Central and South America), where there is no tsetse fly and where transmission is therefore mechanical. Infections with this trypanosome are also encountered in parts of Africa where tsetse flies have not so far been identified.

Pathogenicity

T. vivax can cause disease in all domestic animals except the dog.

In horses the disease is usually chronic and tends towards spontaneous recovery (Hornby, 1949). The clinical symptoms are not very well marked but include anæmia, low fever, inappetence, progressive weakness and emaciation with some œdema of the sheath and subcutaneous tissues of the legs. Experimental infection has been described by Stephen and Mackenzie (1959).

In cattle the pathogenicity varies considerably according to

the strain, the characters of which remain, however, very constant both in morphology and in virulence. Thus, strains which are believed to have been in South America for about two hundred years are still very similar to strains found in Africa to-day. In general, disease in cattle does not differ markedly from that caused by *T. congolense* but usually the parasite appears to be less virulent. In some areas acute cases are recorded.

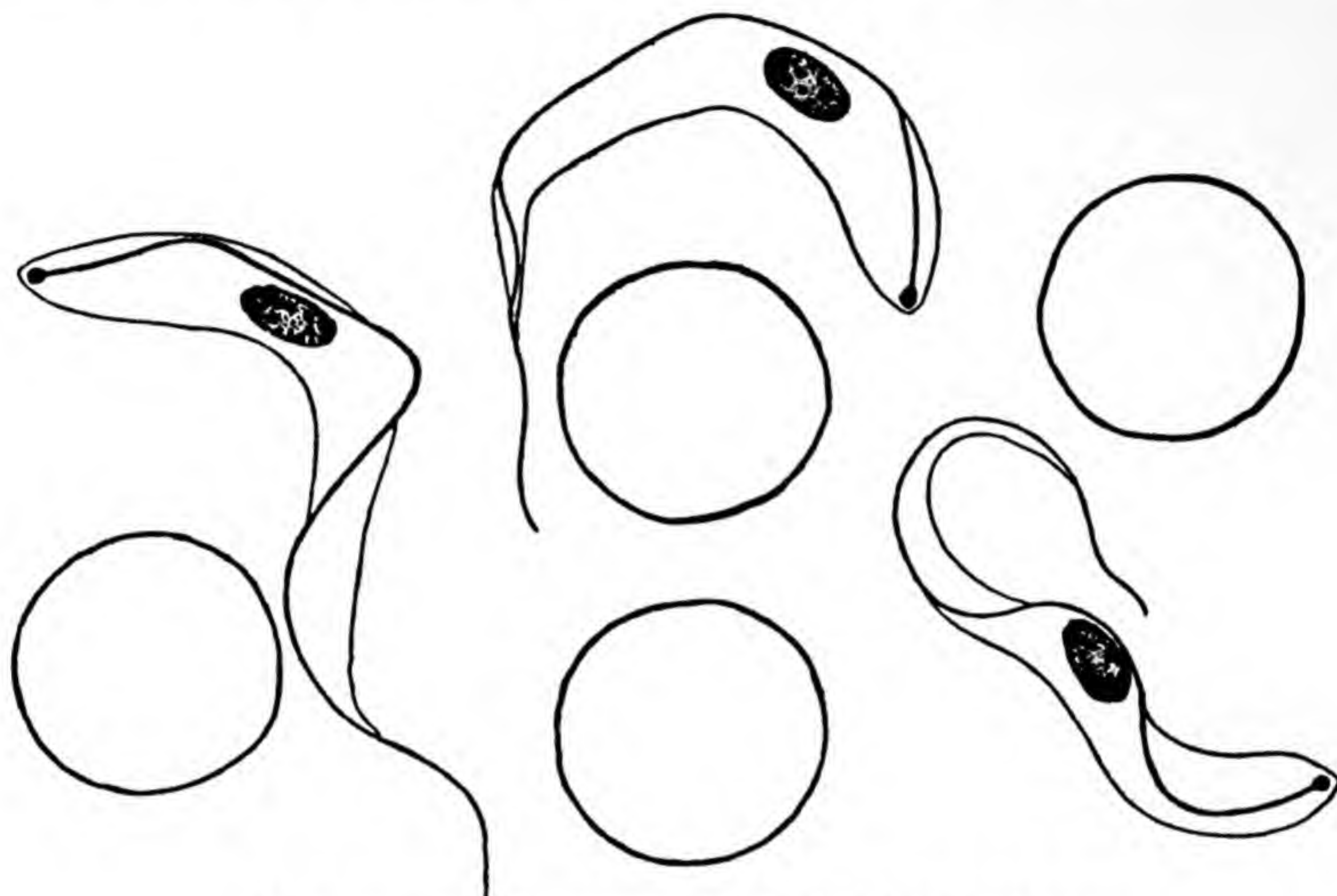


FIG. 5.—*Trypanosoma vivax* in blood of ox.

Sheep and goats. In these, virulence varies as in cattle but in general it is low.

In camels the disease is milder than that caused by *T. congolense*.

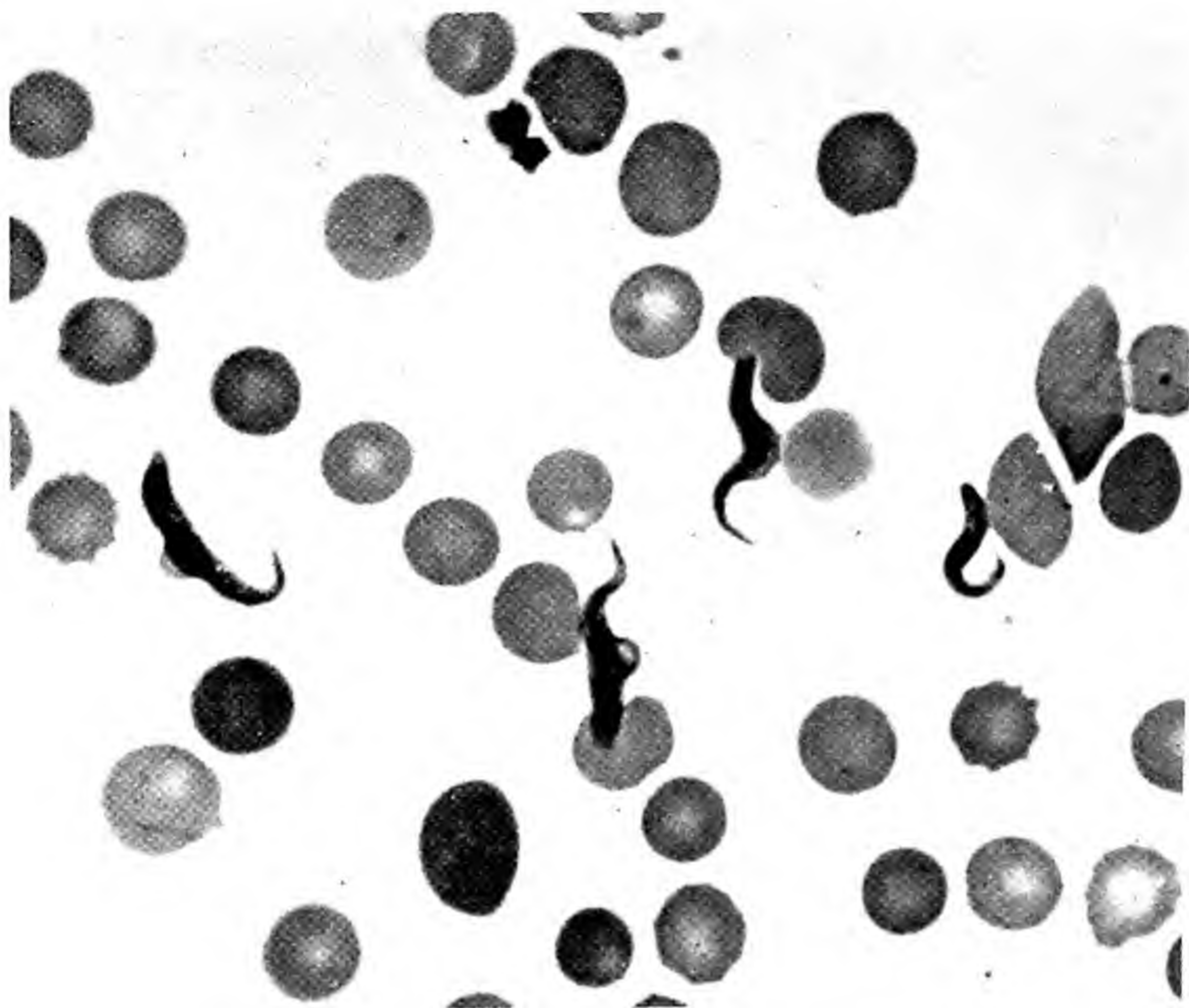
Pigs and dogs are resistant, the dog particularly so.

Desowitz and Watson (1951, '52, '53) showed that a West African strain of *T. vivax* that had been maintained in sheep could be transferred to a white rat but that further passage to white rats was difficult unless a supplementary injection of uninfected sheep or ox's blood followed within 24 hours. Desowitz (1954) later indicated that certain plasma proteins apparently have the ability to facilitate infection.

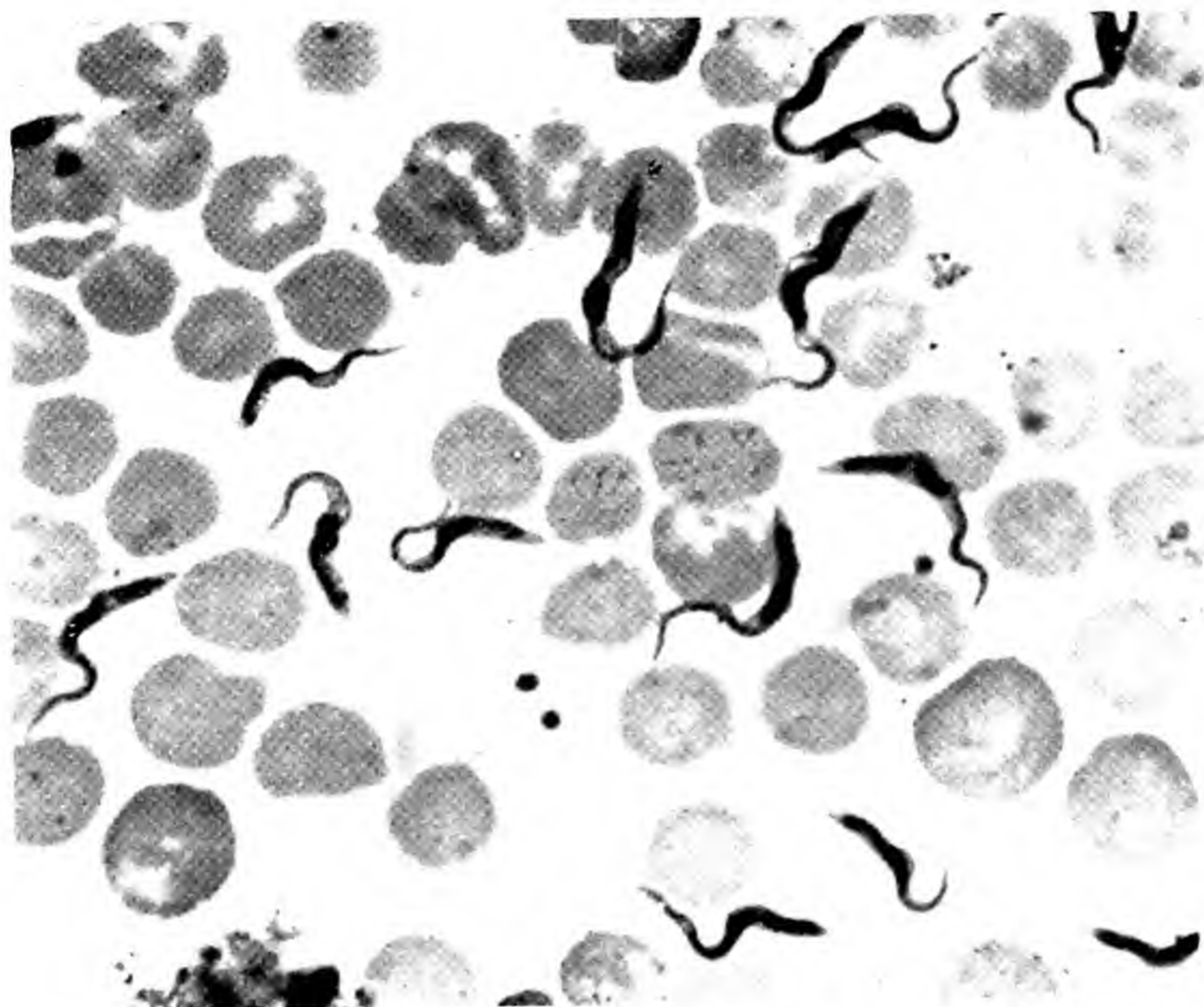
Detection of infection

Swarming infections in the blood are rare and characteristic only of early cases. In the field, the parasite is identified much

FIG. 6.



Trypanosoma congolense



Trypanosoma congolense (Dimorphon type)
Photos : W. Petana

more readily in lymph gland smears. In some chronic cases the parasite may be recovered after subinoculation to a sheep or goat, the prepatent period after inoculation being about seven to ten days. Rodents can be infected.

T. UNIFORME Bruce *et al.*, 1911

This parasite is not readily distinguished from *T. vivax* but is considered by Hornby (1949) to be a good species. It is stated to average about 16 μ in length with a breadth of 1.5 μ to 2.5 μ . The free flagellum is shorter than in the typical *vivax*. A Departmental report from Uganda states that this species is non-pathogenic for goats. Laboratory rodents cannot be infected.

THE CONGOLENSIS GROUP

T. CONGOLENSIS Broden, 1904 (Synonyms *T. pecorum*, *T. dimorphon*, *T. nanum*, *T. montgomeryi*)

Hoare (1959) has concluded that *T. dimorphon* is specifically distinct from *T. congolensis*; a conclusion with which Godfrey (1960) disagrees.

This small monomorphic trypanosome measures about 9 μ to 18 μ in length and is the smallest of the African pathogens. It is in most respects similar to *T. simiae*. The posterior extremity of the parasite is usually blunt, the kinetoplast is some way from the extremity and commonly lies on the margin of the body. The marginal position is not, however, observed in all individuals. Although there is no free flagellum the anterior end of the body may be finely tapered and if the tapered part is folded under the body it may be difficult to detect the cytoplasm extending into the process, which may appear, therefore, as a short free flagellum. The organism is actively motile and in fresh preparations can be seen lashing about among the red blood corpuscles, but it remains in the same field for long periods of time. There is no invasion of the salivary glands of tsetse flies. All laboratory animals are susceptible to infection.

Distribution and pathogenicity

This is probably the commonest and most important of the African trypanosomes and it is the parasite associated most

consistently with the cattle disease known in South Africa as *nagana*. One of the most noteworthy things about *T. congolense* is the existence of a vast number of strains which differ markedly in virulence and which are antigenically distinct. These differences in strain account in part for the very different assessment of the pathogenicity of the parasite which different observers have made. Hornby (1949) describes in detail acute disease which in the ox resulted in death in about ten weeks, chronic disease in

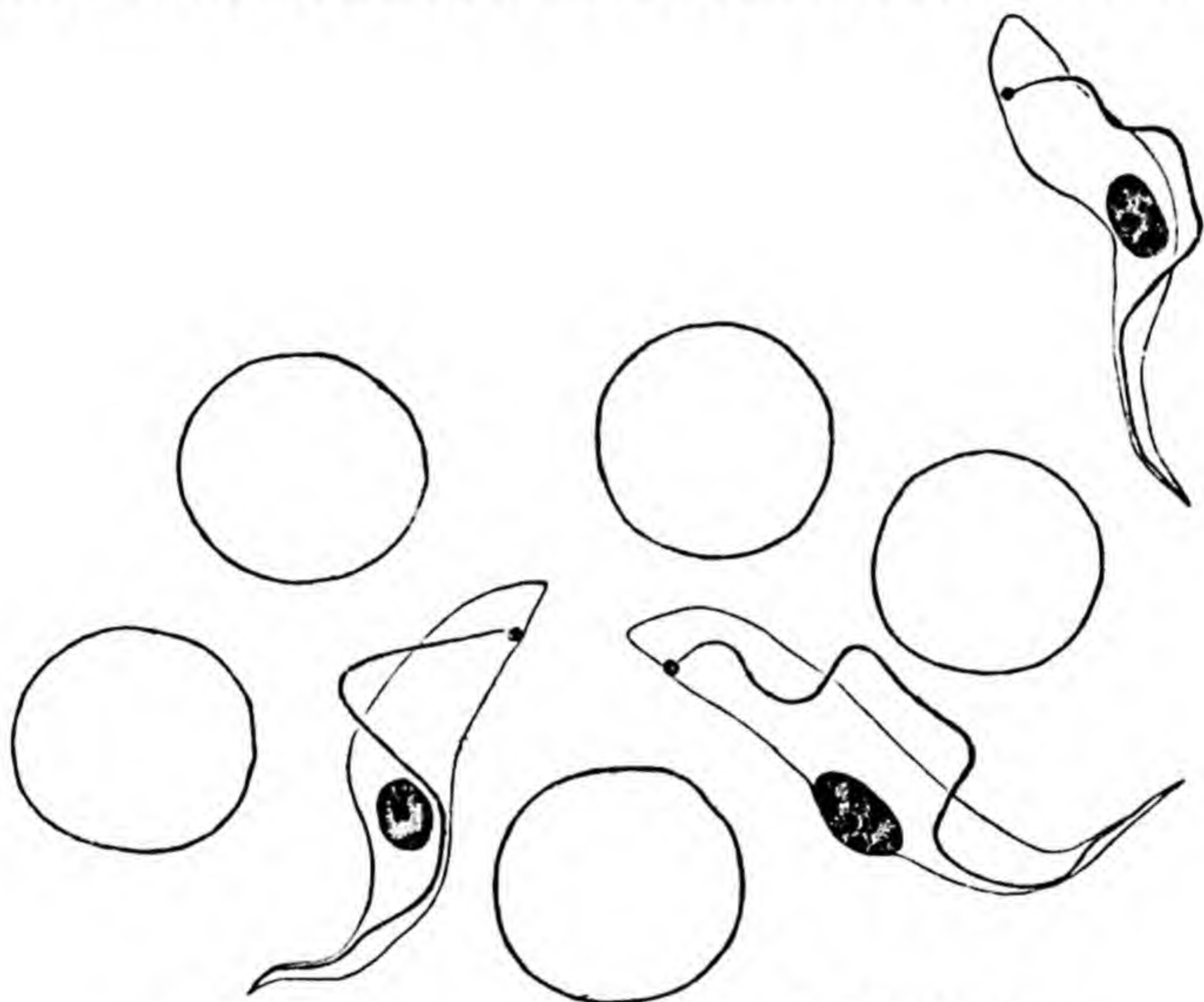


FIG. 7.—*Trypanosoma congolense* in blood of ox.

which recovery occurred in about a year and extremely mild and practically symptomless disease with apparently spontaneous cure.

Besides its common occurrence in cattle, *T. congolense* can cause similar disease in equines, sheep, goats and camels. European dogs are usually seriously affected. Most pigs are at least partly resistant. In 1921 there was a reported occurrence of *T. congolense* in a bullock in the Central Provinces of India. It seems possible, however, that this report was the result of confusion with the commonly occurring *T. evansi*.

Detection of infection

The organisms are more regularly present in the peripheral blood than the other species of trypanosome and can usually be

found by the examination of fresh blood smears. In chronic cases, however, parasites may appear in the blood at rare intervals and daily examination of the blood over long periods may be necessary to reveal it. Sometimes early morning examination of blood from an ear vein may show the presence of parasites. Animal inoculation is ordinarily a sure method of identifying sparse infections but even in guinea-pig, rat or dog some strains

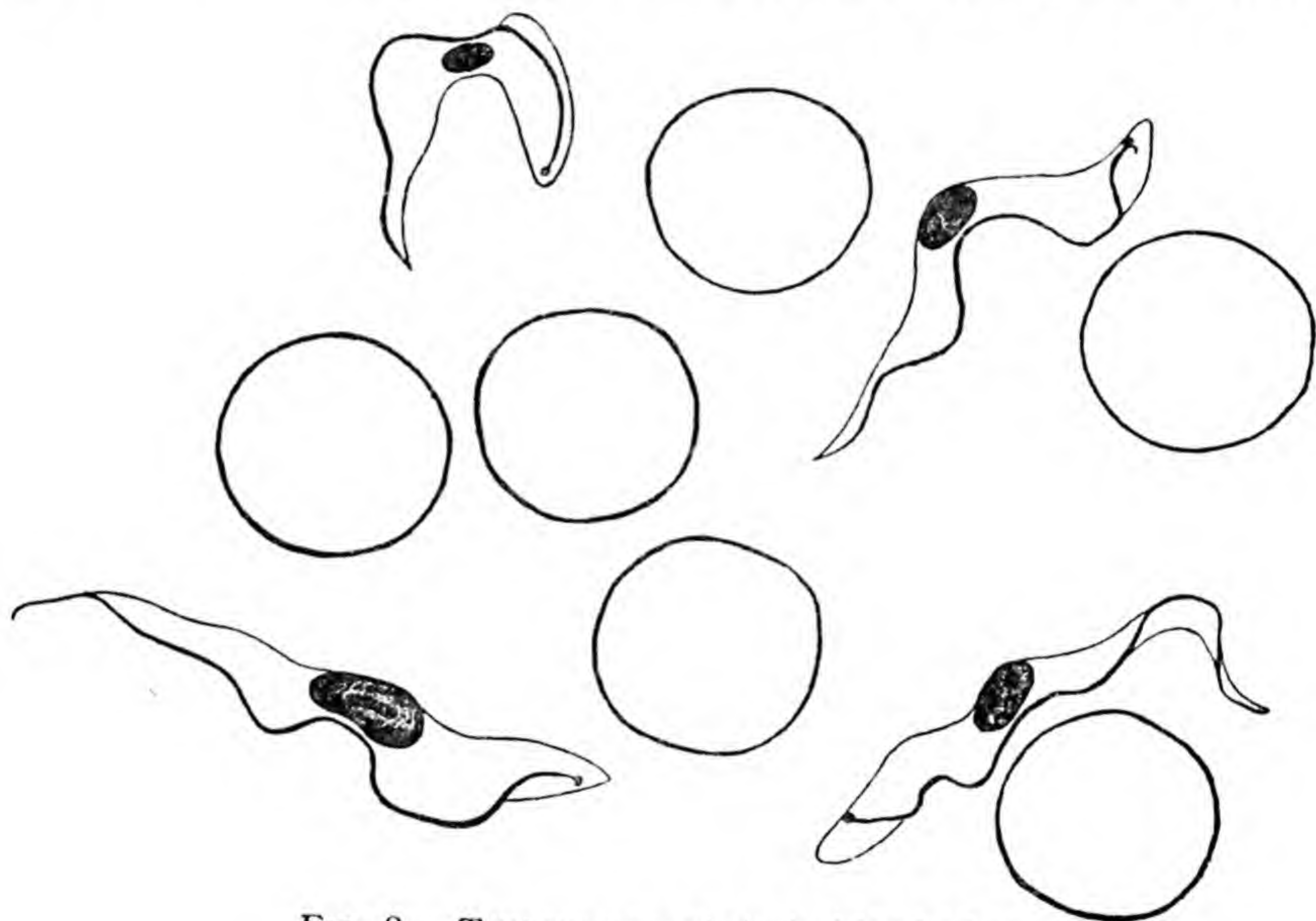


FIG. 8.—*Trypanosoma simiae* in blood of pig.

may occasionally give only fleeting infection of the peripheral blood. The prepatent period may be a month or more and a proportion of animals may show no infection at all. As described elsewhere (Fiennes, 1952) the parasite may persist as a cryptic infection in the heart.

RELATED TRYPANOSOMES

T. ruandæ of the Belgian Congo, *T. somaliense* of Italian Somaliland ;

T. frobeniusi described from a horse in Togoland ; and

T. confusium from Rhodesia are probably identical with *T. congolense*.

TRYPANOSOMA SIMIÆ Bruce *et al.*, 1911

This organism usually resembles some of the longer forms of *T. congolense*. It is about $14\ \mu$ to $24\ \mu$ in length. There is considerable variation both in size and in shape. Like *T. congolense* it often appears to have a short free flagellum.

Pathogenicity

As its name suggests, *T. simiæ* was originally isolated from a monkey in which it can be exceedingly virulent. Since that time, however, it has been shown to be equally virulent for pigs and for camels and in fulminating infections death may occur within a few days. The parasite varies more than any other trypanosome in its pathogenicity and as described by Hornby (1949) remarkable changes in pathogenicity may occur following a single passage under controlled conditions. Disease does not appear to occur in equines, bovines or dogs. In sheep and goats disease which varies between mild and very mild may occur.

Distribution

T. simiæ occurs in Africa following the same general distribution as *T. congolense*.

Detection of infection

In swarming infections identification of the parasite from peripheral blood smears is usually not difficult. In more chronic infections the parasites may be very few. The parasite is most readily differentiated from *T. congolense* by its reaction in the pig. Rabbits can be infected although the parasite is not readily transmitted to laboratory animals.

THE BRUCEI GROUP

TRYPANOSOMA BRUCEI Plimmer and Bradford, 1899

This is a polymorphic trypanosome occurring in long, short and intermediate forms. The long forms are $25\ \mu$ to $35\ \mu$ in length by $2\ \mu$ to $3\ \mu$ in width, and have a long free flagellum $6\ \mu$ to $7\ \mu$ in length. The kinetoplast is situated near the posterior end which is pointed and the nucleus is usually in the anterior half of the body. In a variable percentage of individuals the

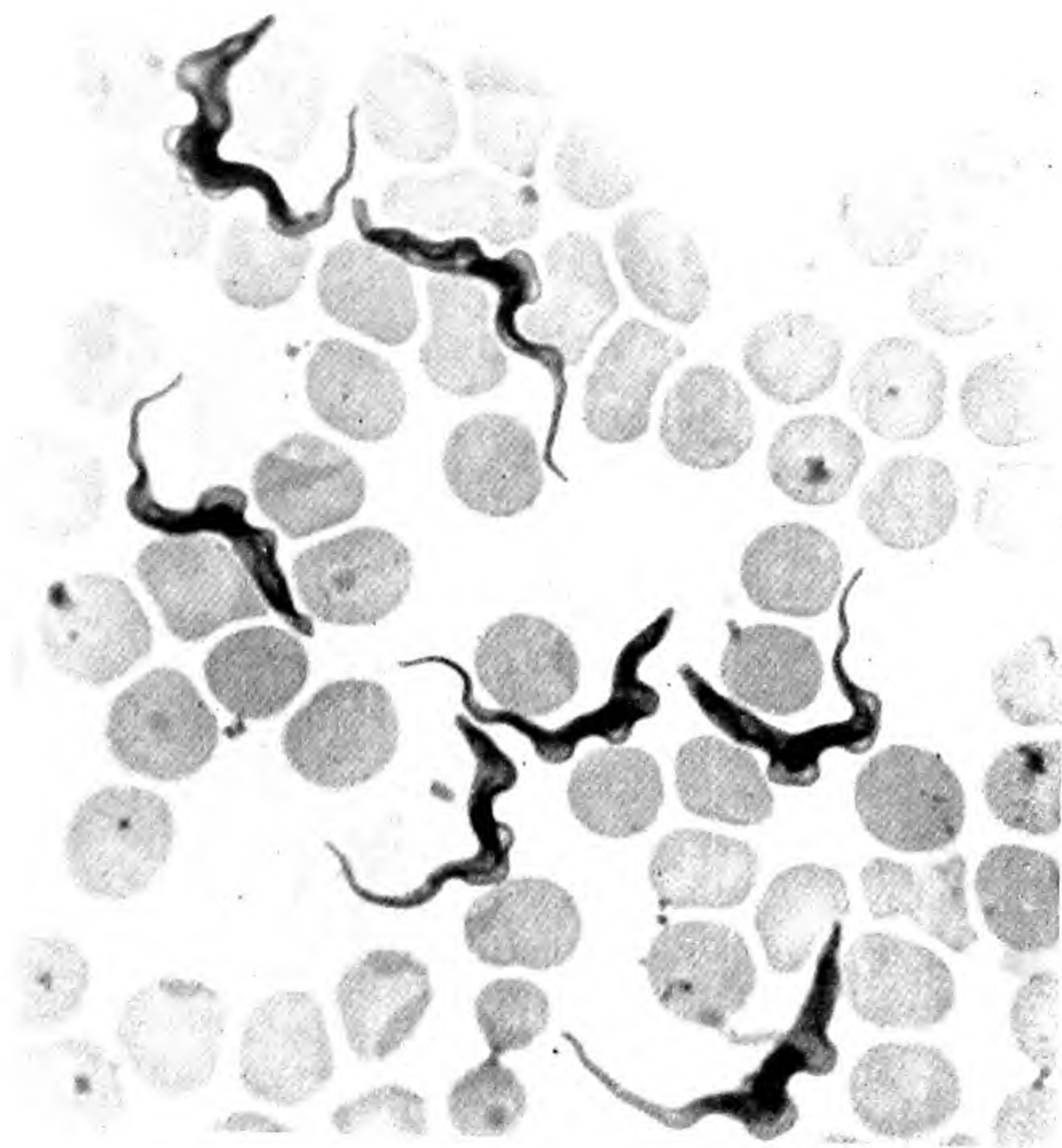


FIG. 9.—*Trypanosoma brucei*. Photo : W. Petana

nucleus is posterior. The short forms average about $15\ \mu$ in length and have no free flagellum and in them the long axis of the nucleus is usually set transversely to the long axis of the body. Both these and intermediate forms may occur together in the blood but one form usually predominates at any one time. All forms have a well-developed undulating membrane. The organisms

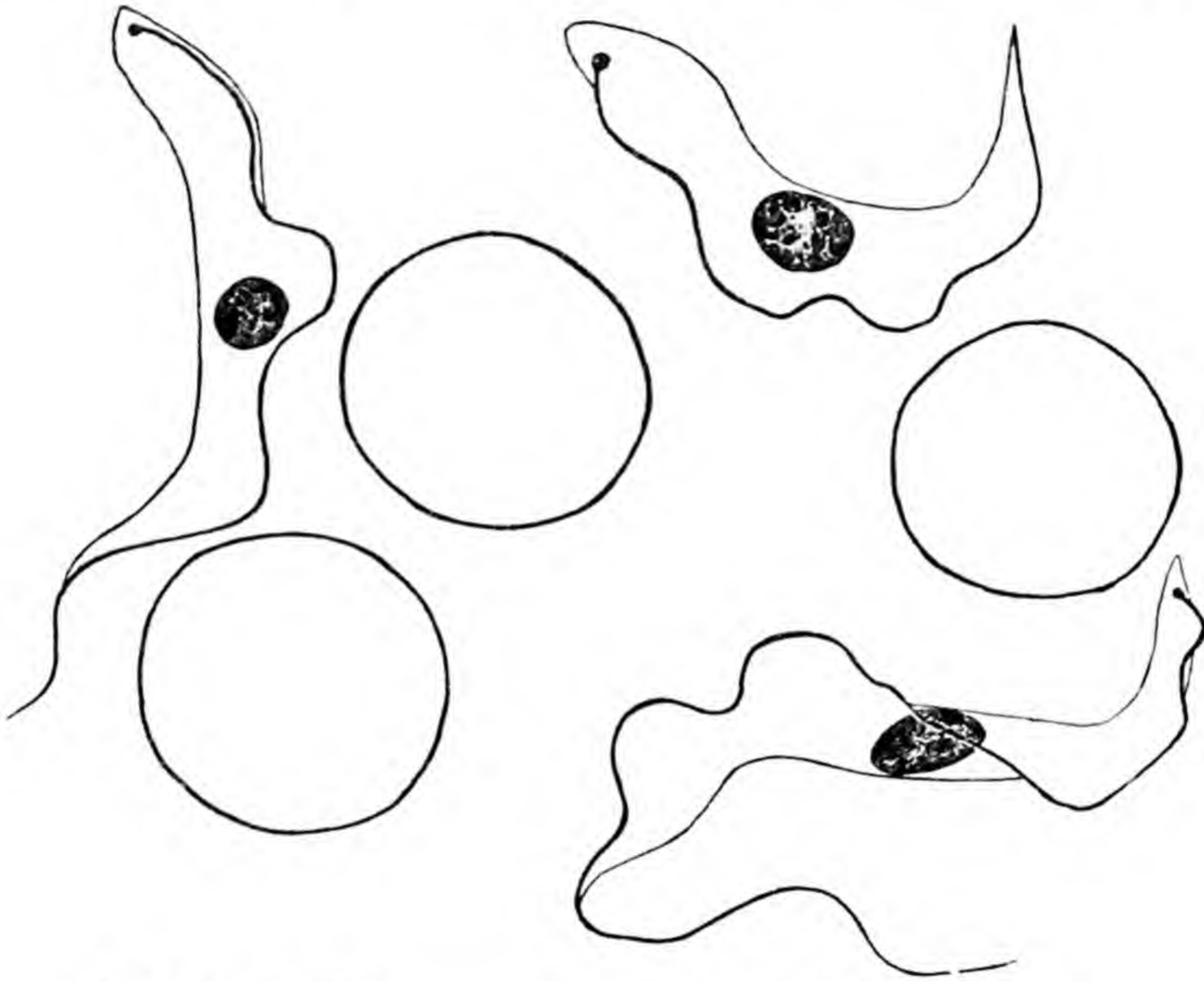


FIG. 10.—*Trypanosoma brucei* in blood of guinea-pig.

are not so active as the *vivax* and *congolense* groups but in fresh preparations cross the field of the microscope with a deliberate undulating movement.

Distribution

This trypanosome has a wide distribution in Africa.

Pathogenicity

Horses, mules and donkeys are very susceptible to infection. A severe disease is also observed in camels and in dogs. Cattle are relatively resistant but sheep and goats suffer as severely as equines. Pigs appear to be resistant, a chronic infection occurring. The parasite is highly virulent for mice and in these a

high proportion of forms showing a posterior nucleus is produced. In rabbits and guinea-pigs a more chronic infection develops.

This organism characteristically causes an extremely serious disease of horses. The untreated disease may be acute or chronic but equines in either event rarely recover. As the disease progresses œdema appears. Sometimes the œdematous areas are confined to the hind legs and genitalia or there may be well defined swellings on the belly. Occasionally the head is involved. In advanced chronic cases there is anæmia with very marked yellowing of the mucous membranes. Fever is intermittent and parasites can usually be demonstrated during the febrile phases. In sheep and goats the disease is similar in character but parasites are more often found in the lymph glands.

In dogs also, the disease is extremely serious and fever may develop in as short a time as five days after infection. In the final stages, nervous symptoms, followed by paralysis, indicate that the spinal fluid has become invaded. A common feature is the penetration of the parasite into all the tissues of the eyes with accompanying conjunctivitis, keratitis and blindness.

Detection of infection

If the disease is acute, parasites can be detected in the peripheral blood. In their absence from this site, however, they may usually be detected in gland smears. It will be remembered that the parasite is very pathogenic when inoculated into laboratory animals. A complement fixation test gives a group reaction for *T. brucei*, *T. evansi* or *T. equiperdum*.

Related species

Trypanosoma gambiense and *T. rhodesiense* are morphologically indistinguishable from *T. brucei*. They differ, however, considerably in pathogenicity, being the two species associated with human sleeping sickness in Africa. The initial stages of the infection are characterised by invasion of the blood stream and the lymph glands. Later the central nervous system is involved. The course of the disease is chronic with *T. gambiense* and acute (with fatal termination) with *T. rhodesiense*. The two diseases are usually geographically distinct in distribution presumably in relation to varying distribution of the species of *Glossina*

which transmit them. *T. gambiense* is transmitted by *Glossina palpalis* and *G. tachinoides*, two species of riverine flies. *T. rhodesiense* is transmitted chiefly by the savannah tsetse *G. swynnertoni* and by *G. morsitans*.

Soltys (1957) has described a series of agglutination reactions between living trypanosomes and the sera of experimentally infected rabbits. Agglutinins appeared to be species specific and *T. rhodesiense* and *T. gambiense* could be separated from *T. brucei*. Wolstenholme and Gear (1958) obtained similar results.

Desowitz (1961) showed that with the loss of polymorphism observed in laboratory strains of *Brucei*-group trypanosomes there was a corresponding loss of antigenic distinction. He suggested that the antigenic difference between species may be a property of the long and short forms. There remained, however, a demonstrable cross-immunity between polymorphic and monomorphic strains. Ashcroft *et al.* (1959) reviewed the infectivity of *T. rhodesiense* and *T. brucei* for wild animals and grouped the hosts into those killed by the two species and those which were relatively or absolutely resistant. The behaviour of *T. rhodesiense* and of *T. brucei* in wild animals was identical. Lehmann (1960) showed that in culture there were both morphological differences between the two species and variations in the average time of survival. On the whole *T. brucei* was more adaptable to culture media than was *T. rhodesiense*.

T. suis Ochmann, 1905, was rediscovered in the Belgian Congo in 1954. It is very pathogenic for pigs. (Peel and Chardome, 1954).

T. pecaui (Laveran, 1907)

This parasite does not appear to differ in any important particular from *T. brucei* and is not here regarded as specifically distinct.

THE EVANSI GROUP

T. EVANSI (Steel, 1885)

This parasite is the cause of "surra" in horses and camels.

This, the first trypanosome to be shown to be pathogenic, is characteristically monomorphic, resembling the long forms of *T. brucei* from which it may have originated. It measures from $18\ \mu$ to $34\ \mu$ in length by $1.5\ \mu$ to $2.5\ \mu$ broad. The kinetoplast

is subterminal, the undulating membrane is well developed and there is a long free flagellum measuring $5\ \mu$ to $6\ \mu$. Movement is undulating and deliberate. There are very occasional stumpy forms without a flagellum. Hoare and Bennett (1938) described Sudanese strains of *T. evansi* in which the kinetoplast was absent.

The following are probably synonymous with *T. evansi*: *T. berberum* which is associated with a disease of horses and camels in Algeria; *T. marocanum*, associated with disease in

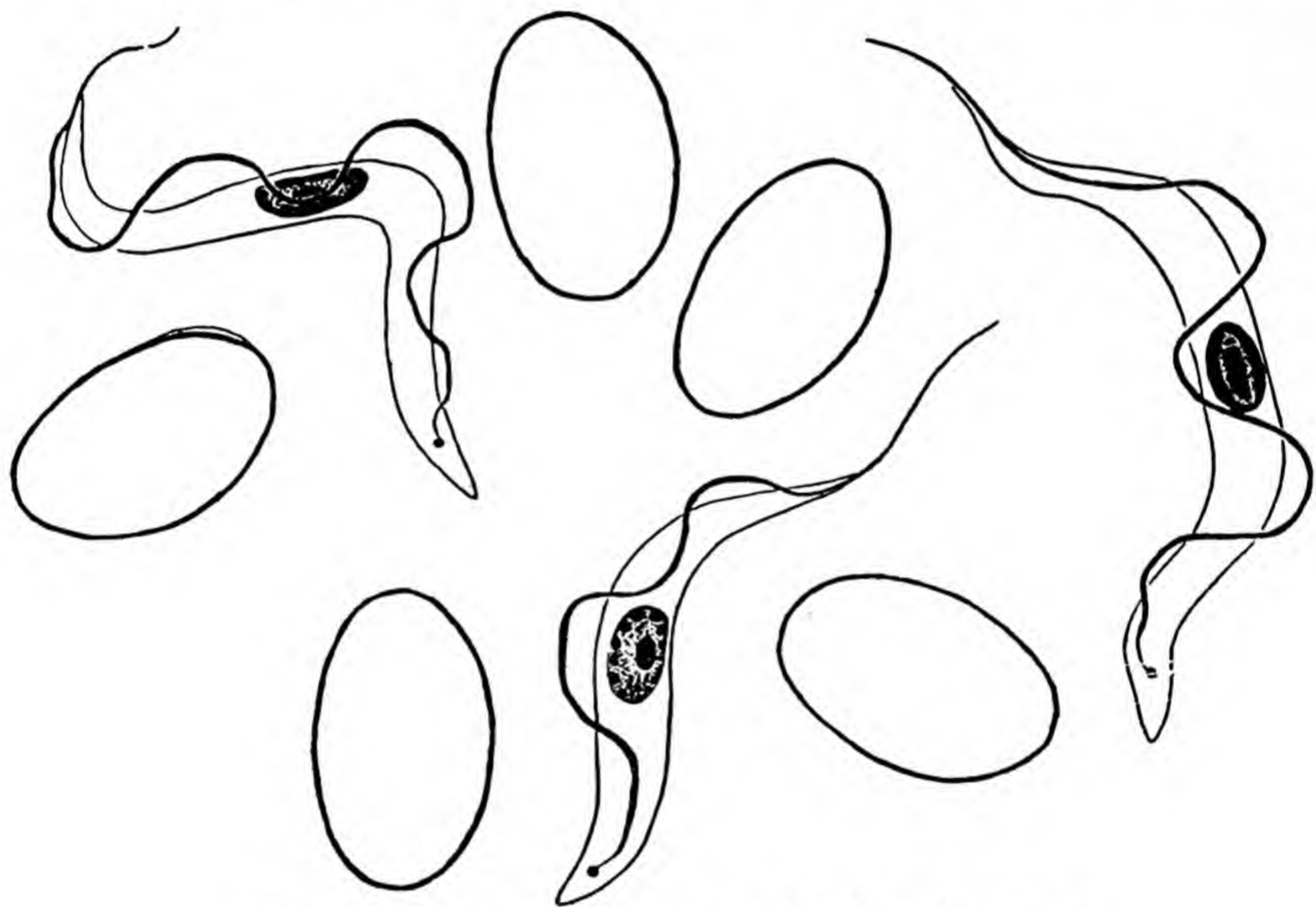


FIG. 11.—*Trypanosoma evansi* in blood of camel.

camels in Morocco; *T. annamense*, causing disease in horses in Annam; *T. soudanense*, causing disease of horses and camels in Egypt and Algiers; *T. hippicum* causing the disease of "murrina" in horses in Panama and *T. venezuelense* causing a disease of horses and dogs in Venezuela known as "derrengadera."

T. equinum which causes the disease known as "Mal de Caderas" in Central and South America, is probably very nearly related but specifically distinct. It differs from the typical *T. evansi* in that the parabasal body (kinetoplast) is usually absent, the axoneme of the flagellum ending in a minute blepharoplast,

but some strains of *T. evansi* from the Sudan show the same character which may also appear in strains maintained in the laboratory.

Distribution

The natural home of *T. evansi* is probably in India but it now has a very wide distribution through the East, through Asia, some parts of Africa and parts of Central and South America. As indicated above, similar diseases occur in many different parts of the world and both the diseases and the organisms associated with them have been given a variety of names.

Transmission

Transmission is mechanical, usually by biting flies particularly of the genus *Tabanus*. In addition *Stomoxys*, *Hæmatopota* and *Lyperosia* have been implicated. Cross (1947) obtained transmission by the ticks *Ornithodoros crossi* and *O. lahorensis*, the ticks not becoming infective until 17 days after the infective feed and remaining infective for as long as 101 days, but there does not seem to be any conclusive evidence of cyclical development. In the Panama area and in parts of South America the vampire bat may act as vector. In India the disease has a seasonal and a regional distribution, being confined to areas where there is a warm climate and where the atmosphere is humid. Such conditions conduce towards mechanical transmission. In horses and dogs the disease is rapidly fatal and it seems probable that in many instances cattle and buffaloes act as reservoir hosts.

Pathogenicity

Cattle are rarely seriously affected by the parasite but they may remain carriers for months. Occasional outbreaks of disease may occur both in cattle and in buffalo.

In South America *T. equinum* affects equines and a native species of rodent, the capybara, may sometimes contract a fatal infection similar to that in the horse although it ordinarily acts as a reservoir host. Elephants and most laboratory animals may contract serious infection.

In horses, the disease of *Surra* is nearly always fatal, death occurring in a week to six months. The condition was first described in India. From India infected animals were moved to Australia, the United States of America and Mauritius and outbreaks occurred in these countries. In Australia and the U.S.A. vigorous policies of eradication were successful. The disease shows symptoms typical of those usually associated with trypanosomiasis. As the disease progresses, intermittent attacks of urticaria occur ; loss of hair and œdema of the legs and dependent parts are characteristic. Conjunctivitis may be present. Donkeys are usually more resistant.

The condition in camels has been well described by Bennett (1933) who developed successful methods of control. The parasites first appear in the blood about a week after infection and a considerable time before there are any marked clinical symptoms. After about a month, progressive weakness and loss of condition become noticeable. Abortion is common. The animal loses its appetite. There are recurring bouts of fever and later œdema. The disease is essentially similar to that in the horse but is nearly always considerably more chronic. In the Sudan, Knowles (1927) described how the disease was well known as "Gufar" and that it was recognised that it was transmitted by *Tabanidae*. Knowles used a formol-gel test for diagnosis and found it reasonably effective. For treatment he used Naganol and antimony (potassium or sodium) tartrate.

In dogs an acute and fatal disease may occur. Ware (1928) described surra in a pack of fox-hounds.

In cattle and buffalo the occasional outbreaks of acute disease are often associated with the introduction of the parasite into a new area. This occurred following its original introduction into Mauritius.

Pathology

The lesions are similar to those of other forms of trypanosomiasis, with marked anæmia and emaciation and with enlargement of the lymphatic glands and spleen. In the case of *Murrina* an enlargement of the liver associated with focal necrosis is described. Leucocytic infiltration of the liver parenchyma occurs. The kidneys also are enlarged and show petechial hæmorrhages

and parenchymatous inflammation. As a result of the kidney disease, blood cells, hæmoglobin, kidney cells and excess protein appear in the urine.

Detection of infection

In horses and dogs infection is usually acute and can easily be detected by the examination of fresh blood preparations or of stained smears. Particularly in camels, however, the disease is often markedly chronic and the organisms are very scanty in the peripheral blood, so that microscopic examination is unreliable. The mercuric chloride test, described by Bennett (1933) in the Sudan has given very reliable results. The complement fixation test gives a group reaction but may be useful if other species of trypanosome are absent.

In cattle the trypanosomes are usually rare in the peripheral circulation and subinoculation of blood into rats or guinea-pigs may give a more reliable result than examination of blood.

Epidemiology

The seasonal and regional distribution of the disease is associated with wet warm conditions when rapid mechanical transmission of the parasite as the result of the interrupted feeding of the blood-sucking vectors is most favoured. The parasite is assumed to overwinter in animals such as cattle and buffalo in which it does not cause fatal disease.

TRYPANOSOMA EQUIPERDUM Doflein, 1901

Morphologically this trypanosome is indistinguishable from *T. evansi*. Usually the parasites are uniform in size measuring from $25\ \mu$ to $28\ \mu$ in length.

Distribution

T. equiperdum occurs in horses in Southern Europe, in Asia and in North and South Africa, causing the disease known as *dourine*. The disease was at one time common in Europe, Canada and the United States but it has been eradicated from most temperate countries. In infected horses the parasites are found in the serous exudate of the œdematous swellings which are characteristic of the disease.

Transmission

Mechanical transmission through biting flies has been demonstrated but the disease is ordinarily transmitted venereally by contact between stallion and mare, the parasites being introduced through abrasions on the external genital organs.

Pathogenicity

The disease naturally attacks horses and asses. Laboratory animals are variably susceptible to infection. Dogs, mice, rats and rabbits may be infected in the laboratory with some strains of the parasite. Cattle appear to be resistant to infection.

In horses, the incubation period is two to twelve weeks but a positive complement fixation test is given three weeks after infection. The disease progresses through three stages. In the first stage, or stage of œdema, there is slight fever and loss of appetite. The genitalia are swollen and œdematous and there is a mucous discharge from the urethra and vagina. There may be œdema of the dependent parts. Loss of pigment occurs in circumscribed areas of the mucous membrane of the vulva and penis. This stage may last four to six weeks before other symptoms develop.

The second stage, or stage of urticaria, is characterised by urticarial plaques which develop on the sides of the body. These plaques are circular and sharply circumscribed and have been described as appearing as if a coin had been inserted under the skin. They are about 3 cm. in diameter. They persist for three or four days and then disappear but may reappear later.

The third stage, or stage of paralysis, is characterised by muscular paralysis developing first in certain groups of muscles, *e.g.* nostrils and muscles of the neck, but later spreading to the hind limbs, causing incoordination of movement, difficulty in backing, etc. Finally paralysis may become complete, the animal being unable to rise.

In many cases, however, particularly in mares, the first stage may cause such slight disturbance as to pass unnoticed and even the later stages may produce few marked symptoms. The mortality rate varies ; in some areas mild strains of the parasite appear to occur, but the disease usually terminates fatally, sometimes after as long as two years. Following experimental

infection resistance persists for nearly nine months (Trumic and Turubatovic 1957).

Pathology

The parasite does not appear to be a true blood parasite and is rarely found in the peripheral circulation. The carcase of an animal which has died of the disease shows great emaciation and there are ulcers on protuberant parts of the body. In stallions the scrotum is often œdematous and the layers of the tunica propria are partially or completely adherent. There is often serous exudation in the epididymis and spermatic cords. In mares, the udder and vulva may be œdematous or indurated. The lymph glands of the genital organs are enlarged. In the spinal cord there are usually no changes visible to the naked eye except occasional punctiform hæmorrhages, but histological examination may reveal foci of perivascular leucocytic infiltration in the grey matter.

The large nerve trunks, especially those of the hind limbs, are either infiltrated with serous fluid or have become invaded with fibrous tissue.

Gelatinous infiltration may extend along the nerve trunks to their roots. Histological examination of the nerve trunks shows cell infiltration and degeneration and atrophy of nerve fibres. In the intervertebral ganglia, particularly of the lumbar region, the nerve cells show degenerative changes, whilst there is a round cell infiltration of the connective tissues. The muscles, particularly those of the hind limbs, show fatty degeneration and round cell infiltration of the intramuscular connective tissue.

Detection of infection and control

The symptoms of dourine are sufficiently characteristic to permit a diagnosis in a typical case without demonstrating parasites. The organisms can often be detected in smears from the mucous membrane of the genitalia or preparations from the fluid of the urticarial swellings, but detection in atypical cases often offers extreme difficulty. Dogs, mice, rats and rabbits are susceptible to infection and develop a blood infection, but the first passage is often extremely difficult to obtain. Some strains, while they may infect some species of laboratory animal,

may fail to infect others, so that in many instances subinoculation fails to reveal infection. It is claimed that intra-testicular subinoculation of the rabbit is a reliable method of detecting infection. To overcome the difficulty of detecting a typical infection the complement fixation test was developed by Watson (1915) and used with entire success as a method of eradicating the disease in Canada. The existence of the disease in South Africa was revealed by this test before the organism had been demonstrated.

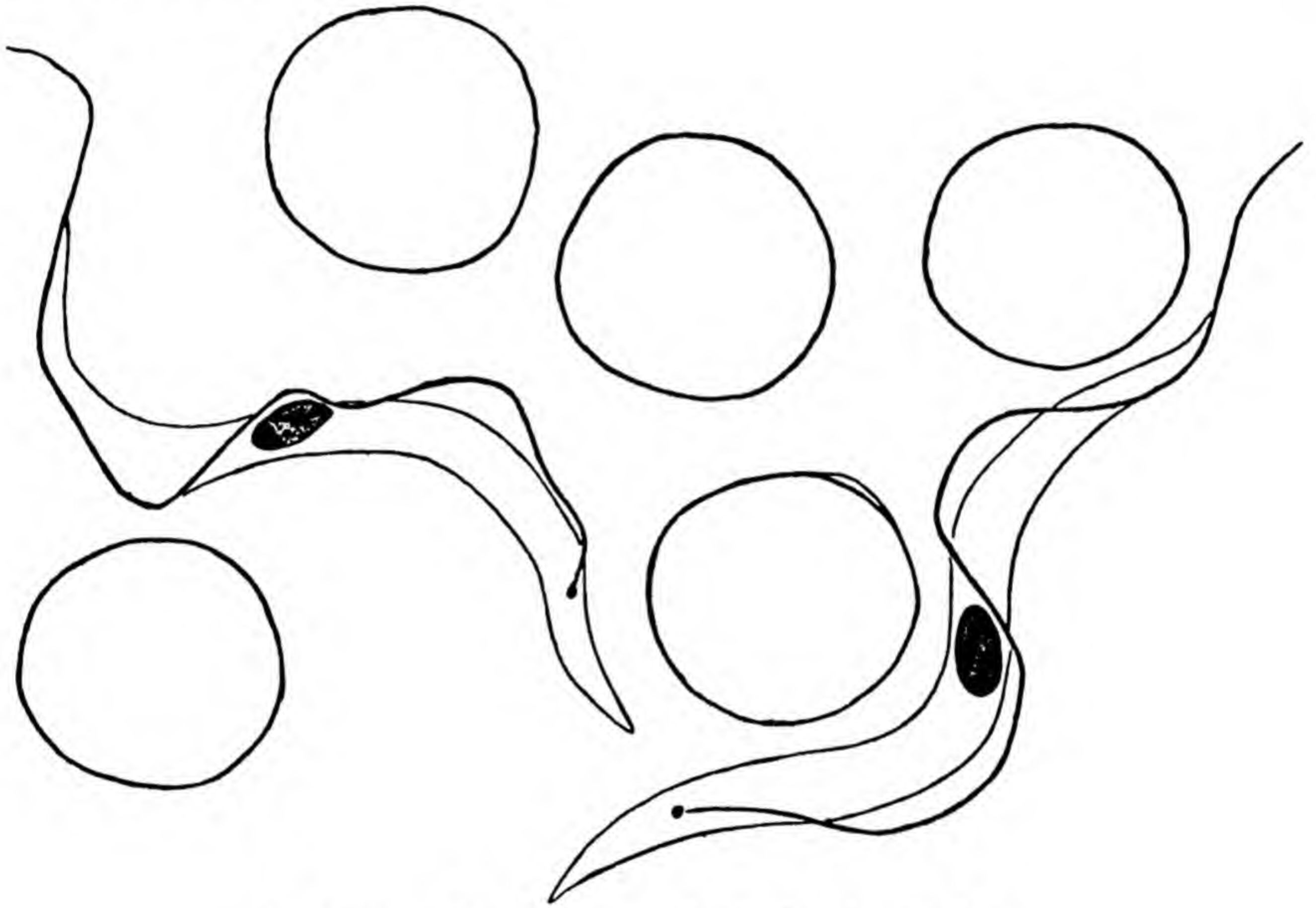


FIG. 12.—*Trypanosoma lewisi* in blood of rat.

The control of both dourine and surra in the U.S.S.R. are discussed by Kazansky (1958). Control is based on routine clinical examination, blood serological tests, treatment of known infected animals and the castration of infected non-pedigree stallions.

THE LEWISI GROUP

T. LEWISI (Kent, 1880)

This parasite is found commonly both in black and in brown rats throughout the world.

Morphology

About $25\ \mu$ in length.

The body of the trypanosome is elongated and there is a drawn-out point at the posterior end. There is a long free flagellum and the undulating membrane is inconspicuous.

Life-history

The parasite is non-pathogenic in the rat. There is a report of the occurrence in man associated with fever. The intermediate host is the rat flea, *Ceratophyllus fasciatus*. When ingested with the rat's blood, development takes place first in the stomach of the flea and later in the rectum. Transmission is through the faeces of infected fleas.

TRYPANOSOMA CRUZI Chagas, 1909

This organism is responsible for Chagas' disease of man in South America.

Morphology

In the blood of the mammalian host the parasite is monomorphic and measures about $20\ \mu$ in length. In the majority of parasites the body is characteristically curved in the form of a sickle or crescent, with a pointed posterior end. The nucleus is in the middle of the body and the kinetoplast subterminal and is unusually large. The undulating membrane is slightly developed. (Fig. 2.)

Distribution

T. cruzi is a parasite of wild rodents, armadillo and numerous other wild and domestic animals in the New World and infects man, particularly young children.

Life-history

The parasite is transmitted by blood-sucking bugs of the family *Reduviidae*. The development of the parasite takes place entirely within the alimentary tract of the bug and the infective forms are developed in the hind-gut (posterior station) whence they are voided with the faeces of the insect. Entrance to the mammalian host is effected through the mucous membranes.

Diamond and Rubin (1958) isolated a strain of *T. cruzi* from a wild raccoon and showed that parenteral introduction of culture material could set up infection in very young pigs, lambs, kids and calves. With the particular strain used there were no clinical signs or pathological changes and there was a low level parasitæmia. In theory, at least, farm mammals in contact with *Reduviid* bugs could become infected and act as carrier hosts.

Diagnosis

Parasites are usually scanty in the peripheral blood, multiplication occurring in the internal organs, where the trypanosomes invade the endothelial cells and in particular the heart muscle. Infection may be detected by xenodiagnosis, by allowing some clean *Reduviid* bugs to feed on the patient and examining their droppings ten days later for the parasites. *T. cruzi* can in addition be revealed in culture medium. A complement fixation test has been reported and the organism can often be demonstrated by subinoculation into a guinea-pig.

Pathogenicity

The pathological effect of Chagas' disease in man can usually be associated with destruction of the reticulo-endothelial cells and degeneration of the heart muscle. In some instances the central nervous system is involved. In dogs the condition often resembles Leishmaniasis with debility, anæmia and splenomegaly.

Treatment

Bayer 7602 was originally produced as a specific against *T. cruzi* but Fulton (1943), working with a virulent laboratory strain of the parasite, found that the drug was not entirely satisfactory. More recently (1945) Eagle reported that γ -(*p*-arsenophenyl) butyric acid was trypanocidal to *T. cruzi* in experimental animals.

T. RANGELI Tejera, 1920

This organism is a parasite of dogs and man in South America and is transmitted by bugs. Development in the salivary glands may occur. It bears a close resemblance to *T. cruzi*, but is a little larger. The kinetoplast is small and placed farther forward in the body.

T. MELOPHAGIUM (Flu, 1908)

This is a parasite of sheep transmitted by the sheep ked. The parasite is rare in the blood but can be demonstrated by culture.

T. THEODORI Theodor, 1928

This organism resembles *T. melophagium* with which it may be identical but occurs in goats. It is transmitted by the hippoboscid fly *Lipoptera caprina*.

T. THEILERI Laveran, 1902

The parasite occurs in cattle, probably throughout the world, but is rarely detected in the blood except by culture. It measures $60\ \mu$ to $70\ \mu$ in length. Occasionally it may appear in swarming infection, particularly following splenectomy. Sudden death simulating anthrax has been reported in such cases. Transmission is believed to be through Tabanid flies. In early infections the organism resembles *T. lewisi*, but as infections become chronic another form appears. This is characterised by the possession of well-marked myonemes and a band-like nucleus. Parasites of this type measuring up to $120\ \mu$ in length have been described under the name of *T. ingens* in African antelopes (Hoare, 1949).

T. CALMETTI Mathis and Léger, 1909 and
T. GALLINARUM Bruce *et al.*, 1911

These are two species that have been described from the fowl. The former is a small species, $36\ \mu$ in length, occurring in South East Asia and the latter is a large form, $60\ \mu$ occurring in tropical Africa. Nothing is known as to their pathogenicity or transmission.

CHAPTER VI

THE CONTROL OF TRYPANOSOMIASIS

THE PRINCIPAL DRUGS USED IN TREATMENT : PROPHYLACTIC TREATMENT : THE CONTROL OF TSETSE FLY

TREATMENT OF TRYPANOSOMIASIS

The principal drugs available are :—

- (1) The antimonials
- (2) The diamidines (including Berenil)
- (3) Surfen C (Congasin)
- (4) Bayer 7602
- (5) Suramin (Antrypol) and its complexes
- (6) The phenanthridinium group (including Prothidium and Metamidium)
- (7) Antrycide

During the past few years there has been a good deal of research into the development of prophylactic drugs such as Antrycide, Prothidium and the suramin complexes.

(1) The antimonials

These include tartar emetic, Antimosan, Stibophen (also known as Fouadin and Neo-antimosan).

Tartar Emetic. In the past, tartar emetic was the most satisfactory of the trypanocidal drugs and it has found considerable favour largely on the grounds of cheapness. It has little effect on *T. brucei* in horses, but is of great value for cattle in the treatment of *T. congolense* and *T. vivax* infection. Many herds were maintained in East Africa, in the fly belts under conditions of light infestation, by regular treatment with tartar emetic. The drug requires skilled administration, as it must be given intravenously in order to prevent tissue necrosis. Tartar emetic given intravenously in doses of one gram at weekly intervals for five to six weeks appears to cure about 65 per cent. of animals (Hornby, 1938). Other animals may relapse but be cured by a second

course of treatment, but a few fail to respond even to repeated treatment. In Uganda, weekly injections of tartar emetic were not found to be curative and were abandoned after an extended trial in favour of the administration on consecutive days of four doses of one gram each, a method recommended by Curson (1923) as a result of observations in South Africa. In weak animals the initial dose should be reduced to 0.5 gm.

The drug is dissolved in saline or water and given in solutions of not greater concentration than 4 per cent. Solutions must be made freshly, as required for use, or excessive toxicity may result.

For *T. evansi* infection in the camel, 200 c.c. of a 1 per cent. solution of tartar emetic has been found to be effective. Drug fastness does not appear to result from the use of tartar emetic.

Antimosan. This drug is issued as a 6.3 per cent. solution and can be given subcutaneously or intravenously, the dose for cattle being 40 to 50 c.c. which is repeated at weekly intervals for five weeks. The drug is less toxic than tartar emetic and is more suitable for the treatment of dogs.

Stibophen (Fouadin, Neo-antimosan) was used extensively in East Africa during the last war. It is closely related chemically to antimosan and has similar properties.

(2) The diamidines

The drugs pentamidine and stilbamidine have not so far been used very extensively as trypanocides in veterinary medicine. Daubney and Hudson (1941) reported the effect on *T. congolense*. Camard (1952) has reported having cured dourine with pentamidine. Ormerod (1952) has reported the development by *T. equiperdum* of resistance to stilbamidine, following treatment with antrycide. *Berenil*, a recently introduced member of the group, has proved very useful in sterilising infections proving resistant to some other drugs. The drug is primarily curative, not prophylactic.

(3) Surfen C (Congasin)

The use of this drug has largely been discontinued because of the intense local reaction which follows its intramuscular use and the great variability of different batches of the drug.

(4) Bayer 7602

This drug was introduced as a specific against *T. cruzi* (but see note in the section on *T. cruzi*).

(5) The Suramin group of drugs

The following are synonyms of Suramin

Antrypol : Bayer 205: Fourneau 309 : Germanin : Naganol : Moranyl.

This group of drugs is of particular value in the treatment of *T. evansi*. In the Sudan, Antrypol has been used on a large scale for the treatment of surra in camels, a single dose of 4 to 5 gm. given intravenously, being relied upon to effect a cure. In some resistant cases, however, antrycide methyl sulphate must be substituted. At present antrycide is very considerably more expensive. In horses and mules a single dose of 4 gm. Antrypol per 1000 lb. body weight usually effects a cure but in some horses this dose may be toxic (Bennett, 1936).

For dourine (*T. equiperdum* in horses) antrypol is similarly effective, about 2 gm. given intravenously and repeated at intervals of 15 days being recommended. The same treatment has been used to protect stallions exposed to infection by the service of mares suspected of infection, but not showing clinical symptoms.

Antrypol is also effective against *T. brucei* in dogs, a dose of about 0.005 gm. per kilo being repeated on the third and on the tenth day.

In the Belgian Congo, Antrypol has been recommended for use against *T. congolense* at a rate of 0.03 gm. per kilo given twice within three days. It should be noted that a high degree of resistance can develop as a result of underdosing with Antrypol.

Suramin complexes

The use of suramin complexes of various trypanocidal drugs (see page 236) which form dépôts of drug has suggested the possibility of very long-term prophylaxis. The ethidium-bromide—suramin complex proved one of the most useful. Unfortunately under field conditions sloughing of the dépôt is likely to occur with a consequent termination of the period of protection.

(6) The phenanthridinium group

Between 1938 and 1944 two related compounds, S.897 and S.1553 were shown to have high activity against *T. vivax* and *T. congolense* although not against *T. brucei* or *T. evansi*. Of these the chloride (S.897) was the more soluble and was cheaper but muscular necrosis was likely to occur at concentrations as low as 1 in 1000. It was largely superseded by dimidium bromide (S.1553) given intramuscularly as a 1 per cent. solution at a rate of 1 mg. per kilo body weight (Wilde, 1949). Higher concentrations of the drug may lead to delayed toxicity with liver disfunction and photosensitisation. This toxicity was investigated by Plowright and Burdin (1952) and by Thorold and Plowright (1952). In 1953 the use of a third phenanthridinium compound, ethidium bromide, was described by Wilde and Robson. This has been found to be less toxic than dimidium bromide (Burdin, 1953) and it is being used extensively at the present time for the control of *T. congolense* and *T. vivax*. The drug is given intramuscularly at a rate of 1 mg. per kilo body weight to cattle. It has been used to cure cattle in which strains of *T. congolense* were showing resistance to dimidium bromide at the 1.5 mg. per kilo level of administration (Karib, Ford and Wilmshurst, 1954). A recent introduction belonging to this group—prothidium—has found wide use, particularly in East Africa and according to the Annual Report of the Kenya Veterinary Department (1960) gives about $1\frac{1}{2}$ times longer protection than does Antrycide Prosalt. Its use may be limited by the fact that it seems to induce drug resistance rather readily.

Metamidium, another of the more recent introductions, is the p-amidino-phenyldiazo-amino derivative of homidium (ethidium) chloride but has structural similarities also with Berenil. It has been used both by itself and complexed with suramin, particularly against *T. congolense* and *T. vivax*.

(7) Antrycide (Quinapyramine)

Antrycide was first described by Curd and Davey (1949) and in the same year Davey made reference at the 14th International Veterinary Congress to its possible application in veterinary medicine. Since that time a good deal of work has been carried out, particularly with reference to the use of the

drug for the control of cattle trypanosomiasis in Africa. The drug is now available for curative use as the methyl sulphate salt. For prophylactic purposes it is used as the pro-salt—a mixture of the methyl sulphate and the chloride. Of the two salts the chloride is only slightly soluble whereas the methyl sulphate is freely soluble.

Two formulations have been used under field conditions ; one containing 1.5 gms. methyl sulphate to 2.0 gms. of the chloride, the other 1.5 gms., methyl sulphate to 10. gm. chloride. The latter formulation is now in general use.

Antrycide methyl sulphate

In the original trials Davey (1950) found that *T. congolense* was controlled at a dose rate of 1 mg./kg. while *T. vivax* needed 5 mg./kg. As much as 12 mg./kg. is usually tolerated by cattle but under average field conditions 5 mg./kg. is the maximum used. In 1956 the manufacturers reduced the recommended dose to 4.4 mg./kg. for *T. vivax*, *T. brucei* and *T. simiae* ; 3.0 mg./kg. for *T. evansi*, *T. equiperdum* and *T. equinum* and 2.2 mg./kg. for *T. congolense*. According to Curd and Davey (1950) antrycide under laboratory conditions appears to be highly effective in controlling *T. congolense*, *T. evansi*, *T. equinum* and *T. equiperdum* in mice. It is moderately effective against *T. brucei*, *T. rhodesiense* and *T. gambiense* but has no effect against *T. cruzi*. In the field the drug is highly effective against acute disease caused by *T. congolense*. Fiennes (1953) considered it to be much less effective against the chronic disease which he considers may be associated with "cryptic" infection. Davey (1957) has questioned this view and in particular does not consider that chronic infection resulting from incomplete treatment or imperfect prophylaxis is qualitatively different from acute infection. Both should respond equally well to adequate doses of an effective drug.

At the generally recommended doses the drug is considerably less effective against *T. vivax* than against *T. congolense* (Garner, 1950). Antrycide methyl sulphate has been used for the control of infection with *T. brucei* in horses but its use is much less safe than in cattle. Two gm., given as a single injection, have been recommended for the treatment of camels suffering from *T. evansi* which fail to respond to other drugs. The drug is excellent for the control of *T. evansi* in horses and cattle.

The pro-salt

The prophylactic use of the pro-salt depends (Davey, 1950) on the slow rate of absorption of the chloride, which forms pockets of drug in the subcutaneous tissues. Davey recommends a dosage of 12 mg. per kilo body weight given every eight weeks. Substantially similar courses of treatment have been shown to give satisfactory results both in West and in East Africa. A single injection appears to give protection for up to 70 days. At the present time herds in the Sudan are reported to have been protected for as long as five years by regular treatment with the pro-salt.

Toxicity of Antrycide

At the recommended doses Antrycide is usually free of side effects but a painful local reaction may sometimes be noted and occasionally there are signs of systemic toxicity (salivating, restlessness and muscle tremors). The local reaction may be controlled by massage of the site and the systemic reaction seems to be reduced if animals are handled quietly and allowed to rest before and after treatment.

Duration of protection given by prophylactic drugs

Field observation suggests a considerable variation in the duration of protection given by individual drugs under different conditions, *e.g.* periods of from 70 to 300 days have been reported for prothidium. (Robson and Milne, 1957 ; Smith, 1959 ; Leach and el Karib, 1960 ; Lyttle, 1960 ; and Robson, 1961.) This variation depends presumably on such factors as the weight of infection to which the cattle are exposed—whether it is mechanical or cyclical, the species of tsetse involved and so on. The length of time for which protection can be expected is therefore inevitably very variable and the need for repeated treatment (usually at 2-3 month intervals) will be largely a matter of local experience.

Drug fastness

Particularly when dealing with labile strains of *T. congolense* the development of drug-fast strains, following long-term treatment without sterilisation seems inevitable. Under field conditions resistance has been shown to develop to the great majority

of the drugs used prophylactically and cross resistance is commonly evinced (Bishop, 1959 ; Wilson, 1950). Some of the complexities of these cross resistant strains have been discussed by Whiteside (1958). Under field conditions it is probably fair to say that resistance can usually be related to treatment at less than the recommended rates. Under all conditions it is, however, an ever present danger. Wenyon (1926) showed that the repeated treatment of mice with atoxyl produced fastness in a strain of trypanosome. If, however, these trypanosomes were transferred to rats they were no longer resistant to the same drug but they were still resistant when transferred back to mice. The problem of drug resistance is clearly linked with the host-parasite balance and with the selection of strains which can resist the defensive resources of the host only under the particular existing circumstances. The danger of fastness to the aromatic drugs was stressed by Yorke (1932) who showed that it might be produced either by repeated doses causing a cumulative effect on a single strain or more likely by the selection of a resistant strain from an originally heterogeneous population.

PREVENTION OF TRYPANOSOMIASIS

(a) By prophylactic treatment

This subject is discussed under the heading of "treatment."

The use of drugs for the control of infection has proved satisfactory under field conditions in Africa and in Uganda, for example, where trypanosomiasis control has been based largely on the use of drugs, considerable areas of fresh grazing have been opened up. To place too much emphasis on drug therapy as a means of controlling trypanosomiasis would, however, be unsound, not only because of the danger of establishing resistant strains in a wide field but also because it does little to diminish the infection as a whole (Weitz, 1961).

(b) By control of the tsetse-fly vector

Existing information does not suggest that the total eradication of African bovine trypanosomiasis is likely to be attained solely by the therapeutic control of the parasite in cattle. No drugs so far described seem sufficiently to combine the virtues of

cheapness and low toxicity with the ability to sterilise the tissues of the host. Protection from infection, by the use of repeated doses of a drug or by the use of a slowly absorbed trypanocidal compound seems invariably to lead to the development of resistant strains of the parasite. A great deal of investigation into the ecology of the tsetse fly and the host-parasite relationships with the trypanosome, has led to a variety of suggested methods of control some of which have shown promise under practical conditions.

Insect repellents and insecticides

The use of insect repellents, to protect cattle in their grazing grounds, has not proved satisfactory, largely because their effective life is so short. The use of insecticides, such as D.D.T., for the control of adult tsetse has yielded promising results although there is the usual difficulty of obtaining a 100 per cent. kill. An air spray may be used in which 5 per cent. D.D.T. is incorporated in 90 per cent. furnace oil with 10 per cent. kerosene and sprayed from the air at about 0.6 gall. per acre. The spray may fail to penetrate thick bush. It is the airborne droplets rather than the surface film which produce the kill. An alternative, very effective but rather expensive method, is the use of insecticidal smokes from the exhaust of an aeroplane. 0.32 lb. of D.D.T. per acre may be very effective in spite of the bush being in leaf.

The use of cattle, sprayed with insecticide, as a means of attracting and killing tsetse fly, has been described by Burnett (1954). The method, using D.D.T., has been used for the control of *Glossina morsitans* in Tanganyika. The results appeared promising but complete extermination was not effected. The use of bait-cattle, *i.e.* unsprayed work oxen which are walked round the edge of areas of bush to attract flies, which are caught by assistants with nets, has been advocated in settled areas where the fly density is very low and where special precautions must be taken to avoid occasional infection.

Deflying houses

Tsetse flies are commonly conveyed for long distances on vehicles—lorries, trains, bullock-carts and cars—and on the boundaries between “safe” fly-free areas and the areas of known

infestation, *deflying houses* have a limited use. These are buildings which are erected on main traffic routes and into which all vehicles are driven while the tsetse flies which they are carrying are removed.

Bush clearing

Clearing the bush, both *discriminative clearing* and *general clearing* have yielded good results in some areas. Of the species of tsetse fly involved in the transmission of trypanosomiasis, *Glossina morsitans*, owing to its enormous distribution and power of living in different kinds of vegetation is of predominant importance. *G. pallidipes* is probably of next importance, at least in East Africa (it is of major importance in Kenya), while the other species, *G. swynnertoni*, *G. brevipalpis*, *G. longipennis*, *G. austeni* and *G. palpalis* are of less veterinary importance. All tsetse flies are dependent on the presence of vegetation and different species have rather rigid preferences. In general, the clearing of bush will eliminate the tsetse fly and a barrier of half a mile gives some measure of protection to grazing cattle. For full protection wider barriers are required and care must be taken to avoid flies being carried across by human activities. In addition, discriminative clearing may eliminate the particular plants which seem essential to the ecology of the fly.

Control of some species, e.g. *G. palpalis*, which lives in linear belts of vegetation along water courses, may thus be comparatively easy. Unfortunately, however, the initial clearing of bush may be only a small part of the control. In tropical countries, regeneration of bush occurs exceedingly rapidly and, unless the soil is sufficiently fertile for permanent settlement to be carried out, the rate of regeneration may be greater than the local population can control.

Methods of smothering the regenerating bush have been studied and there has been some success with *Cynodon sp* ("star-grass") which is also a good pasture grass.

Biological control

Biological control of tsetse has been suggested. *G. morsitans* occurs in many races some of which may be incompatible in breeding; cross-breeding producing sterile young. A method

of control by which male flies of an incompatible race are released in the area where control is required has been considered but does not seem likely to have a practical application. Recently, some attention has been paid to the possibility of control by the release of male flies sterilised by irradiation or by chemical means. There do not seem to be any important parasites of the tsetse fly.

(c) Game Control

The possibility of controlling trypanosomiasis through the destruction of the big game which probably serve as the main hosts of *Glossina* (as well as acting as a reservoir for trypanosomes) has aroused much controversy, both in Africa and outside, and the subject is difficult to approach objectively. There is no doubt that the wild fauna of Africa represents a priceless and rapidly diminishing heritage. On the other hand there is little doubt that wild animals in large numbers and human cultivation and stock rearing cannot co-exist. From the experience in Southern Rhodesia it seems that game destruction results in the disappearance of *G. morsitans*. But *G. morsitans* may be able to survive if cattle are present in large numbers in close association with flies. The species *G. swynnertoni* and *G. pallidipes* seem to depend on game for food but *G. pallidipes* appears to feed mainly on smaller animals. *G. palpalis* feeds on primates, crocodiles and other large reptiles as well as on ungulates. It does not seem likely that extermination of the big game will in all circumstances eradicate tsetse fly, although it may reduce their numbers. Probably a compromise will prove the final solution, whereby national parks will be isolated from human settlement by barrier clearings, by sterile zones in which all game will be shot or driven away and where all forms of traffic will be rigidly controlled.

CHAPTER VII

THE ORDER POLYMASTIGINA

TRICHOMONAS : HEXAMITA : GIARDIA

THE majority of the members of this Order inhabit the digestive tract of animals. There are 3-8 flagella and one or more nuclei. Often a cytostome is present and a supporting rod—the axostyle is characteristic of the group. There is a clear distinction between the sub-Orders the *Monomonadina* (with one nucleus) and the *Diplomonadina* (with two nuclei). Four genera of *Monomonadina* occur in domestic animals and birds and may be encountered in disease investigations.

(1) *Trichomonas*, with four to six flagella, an undulating membrane and well-developed axostyle.

(2) *Eutrichomastix*, resembling *Trichomonas*, but with no undulating membrane. An example is *Eutrichomastix gallinarum* which occurs in the cæca of fowls. Other species occur in the rumen of cattle and the cæcum of guinea-pigs but none of these organisms appears to be pathogenic.

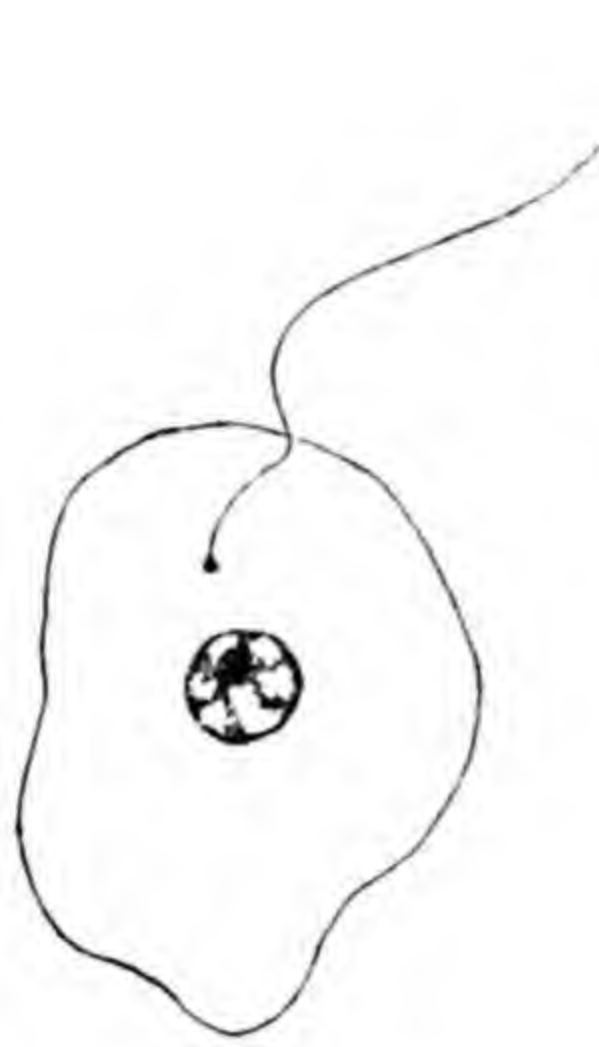
(3) *Protrichomonas*. There are three anterior flagella and the axostyle is replaced by two fibrils which pass backwards on either side of the nucleus. Example, *Protrichomonas anatis* of the cloaca of the duck. This is non-pathogenic.

(4) *Cochlosoma*. This genus resembles *Protrichomonas* except that there are six anterior flagella and a depression occurs on one side of the body, resembling the sucking disc of *Giardia*.

The majority of these organisms inhabit the intestinal tracts of vertebrates and are most commonly encountered in diarrhœtic fæces but there is little or no evidence of pathogenicity. As a rule they form cysts which are passed out with the fæces of the host. The cysts are thin walled so that the nucleus and the principal structures of the parasites can be seen.

The genus which is of major veterinary importance—*Trichomonas*, does not encyst.

Two genera of *Diplomonadina*—*Hexamita* and *Giardia* are of veterinary interest.



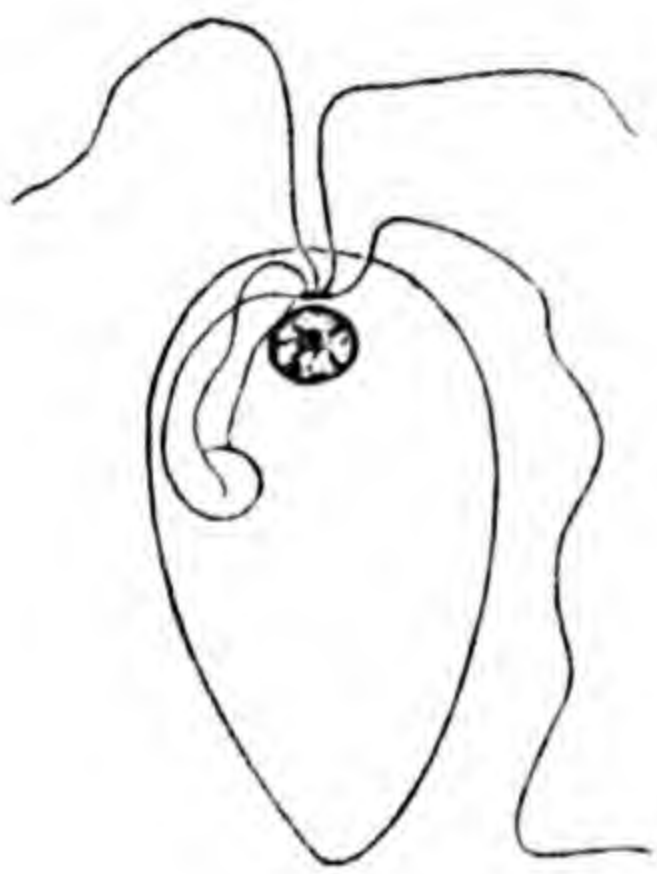
Histomonas



Embadomonas



Cercomonas



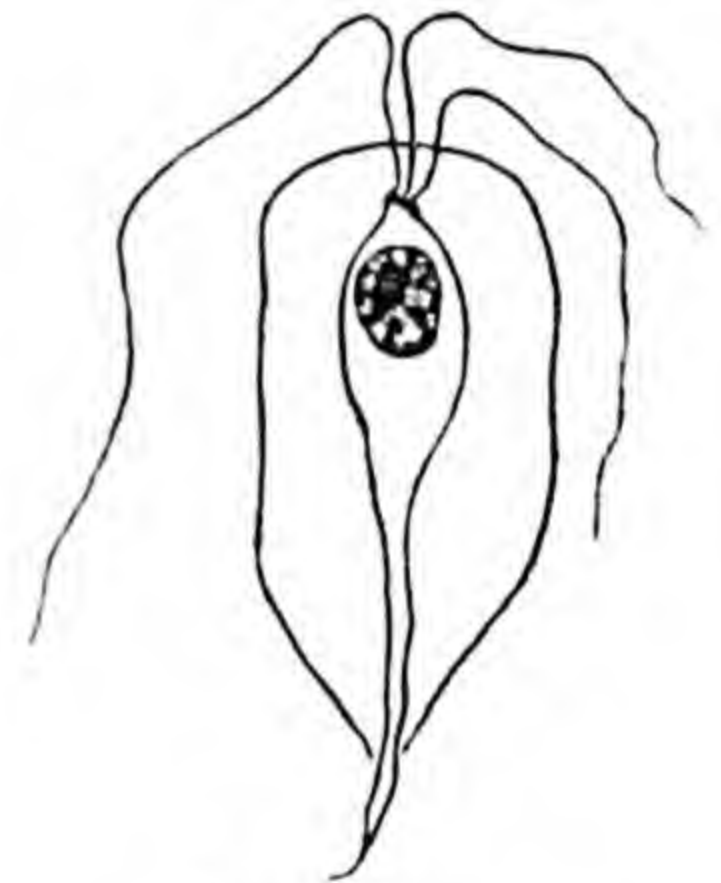
Chilomastix



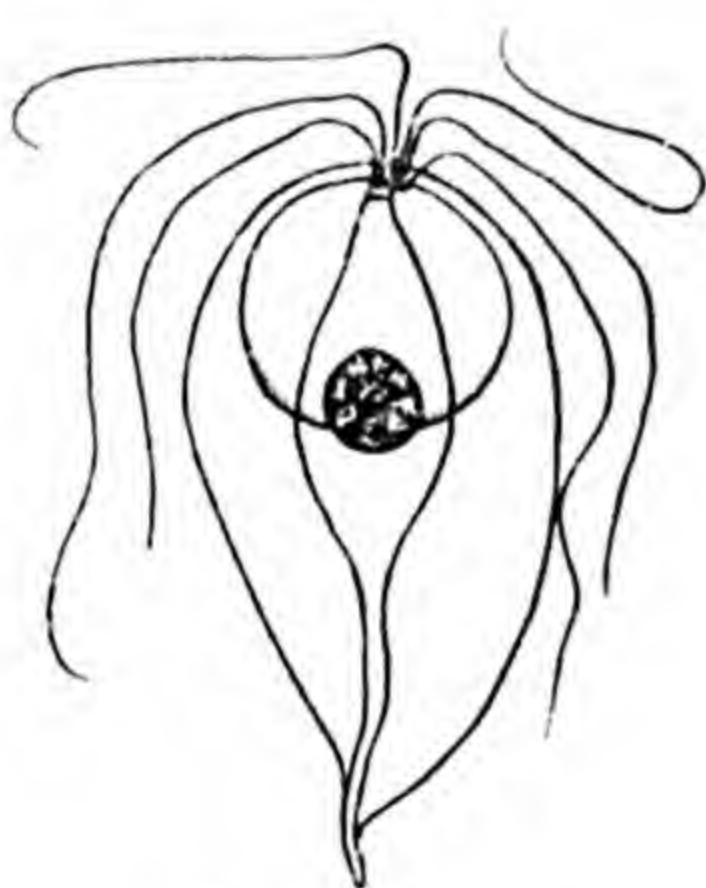
Trichomonas



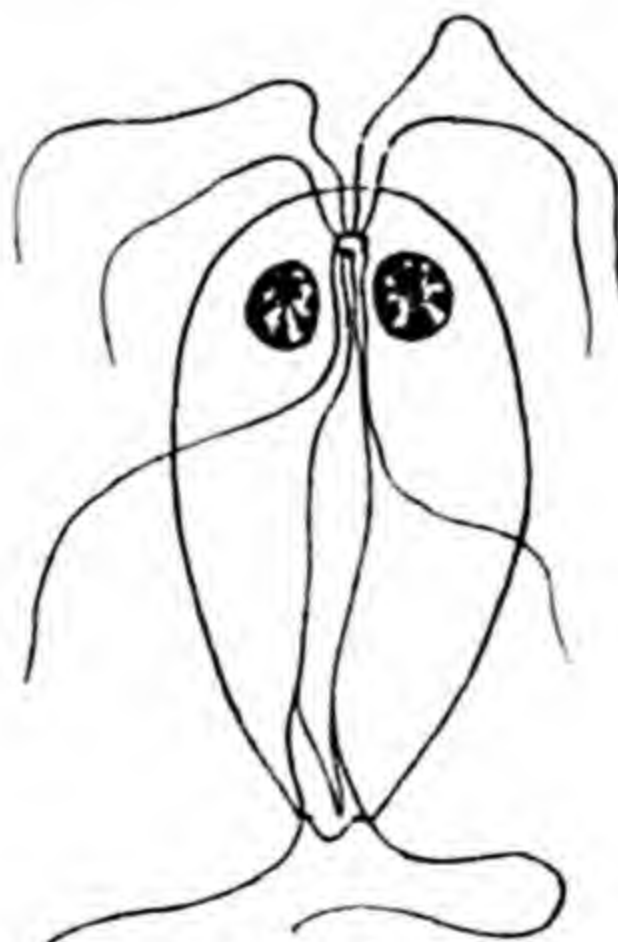
Eutrichomastix



Protrichomonas



Cochlosoma



Hexamita



Giardia

FIG. 13.—Flagellates with more than one flagellum (diagrammatic).

Genus *TRICHOMONAS* Donné, 1837

Various species of *Trichomonas* may be found particularly in diarrhœtic fæces of man and domestic animals. Another species, *T. vaginalis*, is associated with vaginitis in women. The following are the species which are of major veterinary interest :—

TRICHOMONAS GALLINARUM Martin and
Robertson, 1911

This organism causes avian trichomoniasis of the lower intestine.

Morphology

The organism is usually pear-shaped, measuring about $6\ \mu$ to $8\ \mu$ in width and about $9\ \mu$ to $12\ \mu$ in length. There are four anterior flagella and one trailing flagellum which is free posteriorly. The latter arises from the anterior blepharoplast complex and passes posteriorly as the marginal filament of the undulating membrane. Anteriorly, there are two blepharoplasts. The nucleus is near the anterior end of the body and has chromatin distributed on the nuclear membrane. The parabasal body lies at the base of the undulating membrane and appears as a row of granules. The cytostome is usually barely apparent. The axostyle is slender and projects from the posterior margin of the body at about the same point as the trailing flagellum. No cysts are formed.

Species susceptible to infection

Turkey, domestic fowl, guinea-fowl.

Symptoms

The disease is characterised by the appearance of liquid pale yellow cæcal diarrhœa, ruffled feathers, loss of appetite and a very apparent loss in weight. In many reported cases there appears to have been confusion with Histomoniasis, a disease which Trichomoniasis clinically to some extent resembles.

Pathology

Lesions appear in the lower intestine. Allen (1941) administered cultures of an organism resembling *T. gallinarum* to young

turkeys and produced an entero-hepatitis very similar to that caused by *Histomonas meleagridis*. This work has not yet been repeated and there is little other information available on the pathogenicity of *T. gallinarum*.

Transmission and epidemiology

Little is known. McLaughlin (1957) has studied the survival of *T. gallinarum* in chicken faeces.

Treatment

2-amino-5-nitrothiazole, as recommended for *T. gallinæ*, is likely to prove effective.

TRICHOMONAS GALLINÆ (Rivolta, 1878)

This causes avian trichomoniasis of the upper intestine and is found particularly in pigeons. *T. columbæ* is a synonym.

Morphology

In general size and shape the organism resembles *T. gallinarum*. The marginal filament of the undulating membrane extends down one side to a point about two-thirds the total length of the body and does not terminate in a trailing flagellum. The axostyle passes through the long axis of the body and protrudes for a short distance posteriorly.

Species susceptible to infection

The organism has been found in natural infection in the upper intestine of pigeons, turkeys, chickens (Rac and Hreczko 1960), and a number of other species of birds. It is of greatest importance when causing disease among pigeons. Locke *et al.* (1961) have reported its occurrence in the ground-dove *Columbigallina passerina*. Honigberg (1961) passaged a strain through mice.

Symptoms

In turkeys and chickens the symptoms are not pathognomonic of the disease. In pigeons, infection is often symptomless and in fact the great majority of domestic pigeons carry the infection.

Occasional acute outbreaks of disease occur, however, particularly in young birds. These outbreaks are characterised by rapid wasting followed by extreme weakness and death. Mesa *et al.* (1961) have described the histopathological changes following infection with a strain of *T. gallinæ*.

Pathology

Strains of *T. gallinæ* vary very considerably in virulence. With a virulent strain Stabler and Engley (1946) found that the earliest visible lesions appeared in the mouth cavity, most commonly in the soft palate. Œsophagus, crop and proventriculus also were involved. The digestive tract posterior to the proventriculus is not affected. The liver, however, is frequently involved and, apparently by extension, the lungs, the serous surface of the intestine, the pancreas and heart.

The first sign of infection in the pigeon is the development of a small yellowish area on the oral mucosa 3 to 14 days following inoculation. This increases in size and other similar lesions form until the entire œsophagus and trachea may be blocked. The tissues in the roof of the mouth are frequently invaded, producing large caseous masses. Sometimes the bones in the floor of the skull are invaded. In the crop and œsophagus are small white nodules, the contents of which are caseous in nature and may be expressed on pressure. Later, circumscribed necrotic areas, yellowish grey in colour, appear. These are firmly attached to the mucosa and may occlude the lumen of the crop and œsophagus. The gizzard and small and large intestine never bear internal lesions.

Transmission and epidemiology

The exact way in which *T. gallinæ* is transmitted to chickens and turkeys is not known. The organism is very delicate with little ability to withstand conditions outside the body. Moreover, it appears unlikely to be passed in a viable condition with the fæces. Drinking water is probably the sole avenue of infection (Stabler, 1954).

In pigeons the organism is passed directly from the carrier mother to the newly hatched squab in the "pigeon-milk" with which she feeds it. Transmission is therefore direct and certain.

A pigeon kept in isolation may retain infection for a year or longer.

Diagnosis

Diagnosis is made on the occurrence of the characteristic mouth lesion, and by the identification of the organism. *T. gallinæ* is a very easy organism to culture (Stabler, 1954) and according to Joyner (private communication) culture in Glucose-broth-serum medium is one of the best means of demonstrating the parasite in post-mortem material. Usually, however, the organisms are present in very large numbers in the mouth and crop contents.

Treatment

Stabler and Melletin (1953) described the very successful use of 2-amino-5-nitrothiazole for the control of *T. gallinæ*. It is recommended that pigeons should be given about 30 mg. per kilo body weight daily for seven days. This appears to remove all parasites even from symptomless carriers.

Stabler (1957) further described the effect of furazolidone. The drug was given in gelatin capsules which were inserted into the crop of the pigeon daily for seven days. At the rate of 35-200 mg./day the drug proved toxic. There were convulsions and much weight loss. There were signs of toxicity at 25 mg./day but the birds survived a total of 175 mg. At 10 mg./day all the birds survived a total of 70 mg. At this non-toxic level the drug did not eliminate the parasite but it seemed to control mortality. At 25-35 mg./day the parasites were usually eliminated.

TRICHOMONAS ANSERI (Hegner, 1929)

This species occurs in the cæca of geese and has been experimentally transferred to chickens. The body is oval in shape and averages about $8\ \mu$ in length by $4.7\ \mu$ in width. There are four anterior flagella arising from two anterior blepharoplasts. A marginal filament passes posteriorly, bordering the undulating membrane and terminates in a trailing flagellum. The axostyle protrudes considerably at the posterior end.

This species is probably non-pathogenic.

TRICHOMONAS FÆTUS Riedmuller, 1928

Trichomoniasis caused by *T. fætus* is a specific venereal disease of cattle characterised by infertility and by abortion.

Morphology of the parasite

The causal organism is pear-shaped and slightly larger than a polymorphonuclear leucocyte—sometimes it may be up to

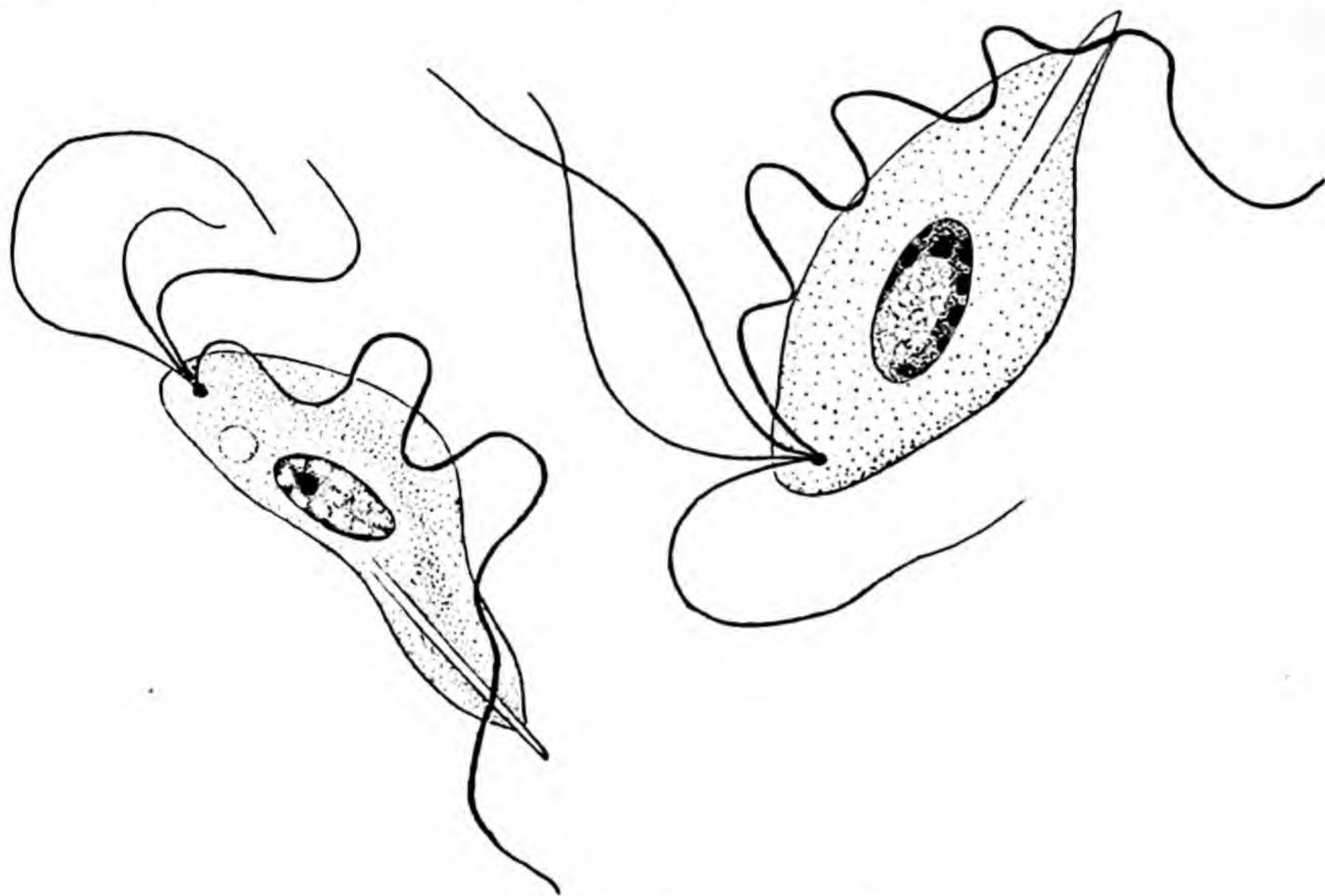


FIG. 14.—*Trichomonas fætus*.

24 μ in length. It has four flagella, three of which project from the anterior end and one which also originates anteriorly but which extends backwards along the edge of the undulating membrane, being continued posteriorly as a free flagellum. There is a supporting axostyle which runs the whole length of the organism and which may or may not project posteriorly. The single nucleus is situated anteriorly and is surrounded by cytoplasm which usually contains several vacuoles. Ordinarily *T. fætus* is actively motile, progressing by vigorous movements of its anterior flagella in a characteristic jerky fashion. Particularly when at lower temperatures movement becomes sluggish and the organism may progress by movement of the undulating membrane. Rounded

forms showing little or no movement are sometimes found in fresh vaginal discharges from infected cattle.

No processes of conjugation or encystation have been seen. Outside the body the organism can survive for a few days in mucus or in the aborted foetus but it is rapidly killed by dryness.

Species susceptible to infection

Natural infection is confined to the bovine. In the bull the preputial cavity is the predilection site. In the cow, the parasites occur throughout the genital tract. They may invade the foetus and are particularly common in the foetal stomach. The distribution of the disease is probably world-wide.

Transmission

Ordinarily the disease is transmitted by coitus, but infection may occasionally be transmitted on instruments, or on hands and arms during veterinary examination or through the use of semen from an infected bull during artificial insemination.

Symptoms and pathology

(a) *In the female.* The original site of infection is the vagina whence the organism attains the uterus via the cervix. One symptom of the disease may be a vaginitis associated with granular lesions on the floor of the vagina anteriorly.

The first symptom in an affected cow is a periodic vaginal discharge which is particularly likely to occur one or two days prior to the expected time of oestrus or a few days after service. The discharge may be so slight as to escape attention or may be a copious amount of thin greyish-white fluid. Following service, there may be a prolonged anæstrus with pyometra. This arises from the early death of the foetus *in utero* with retention of the corpus luteum and the cervical seal of pregnancy. The foetus is not expelled but becomes macerated while uterine secretions accumulate. The presence of the cervical seal hinders the escape of exudate, which may, however, be forced out by unusual pressure caused by the movements of the animal.

Under other circumstances, abortion occurs with expulsion of a small foetus not later than the 16th week. This early abortion

is one of the most characteristic aspects of the disease. Later abortion (after 6 months' pregnancy) is very rare.

(b) *In the male.* Symptoms in the bull are not always apparent. Often there is only a slight swelling of the prepuce. Some pain may be exhibited at micturition or at service with the result that the bull becomes disinclined to serve. In early infection, a discharge may be apparent, associated with small red nodules in the mucous surfaces of the prepuce. As the condition becomes chronic the swelling and pain disappear.

Resistance and immunity

There is, in cattle, no evidence of a natural resistance to infection with *Trichomonas foetus* but it is common clinical experience that a cow rarely aborts more than once and very rarely more than twice. Self-cure from the clinical aspect of the disease ordinarily occurs in cows but it is not certain that a sterile immunity always follows. The bull appears to remain permanently infected. If, in the cow, however, the uterine mucosa has been badly damaged following retention of the foetal membranes with metritis, the animal may be rendered permanently sterile.

Diagnosis

(a) *By herd history.* The typical history is that of increasing infertility the commencement of which can be related to the importation of new stock—usually a bull. The early abortions which are so characteristic of the condition usually pass unnoticed, however, and the farmer is aware of the failure to conceive only after repeated services. Relating the outbreak to the arrival of a particular animal will suggest the need for careful individual examination.

(b) *Demonstration of the organism.* Cultural methods, as described later, usually give a better result than does direct examination. Allsop *et al.* (1961) showed that kidney tissue monolayers give good primary cultures and appear slightly more sensitive for the detection of *Trichomonas* than is the conventional G.B.S. medium. Trichomonads are as a rule most easily found in the stomach of an aborted foetus, in the amniotic and allantoic fluids and in the discharges which accompany the abortion. In the cow, discharges are best collected 2 or 3 days before the time

at which œstrus is expected. Diagnosis may be made with washings from the vagina and uterus, with mucus from the cervical area of the vagina, with uterine pus or with pieces of placenta. Mucus from the vagina is best collected in a sterile glass tube using the method later mentioned for the collection of mucus for serological examination.

In the bull, preputial washings or swabs taken from the preputial cavity are the best source of material. Care must be taken to avoid the introduction of fæcal material which may contain coprozoic organisms. Material is best collected by introducing approximately 100 c.c. of warm 0.85 per cent. saline into the preputial cavity by means of a catheter. After the injection of the fluid the catheter is withdrawn, the preputial orifice closed manually and the saline massaged well into the sheath. The saline is afterwards drained into a wide-necked jar.

Hammond and Bartlett (1943) reported that the diagnosis of bovine Trichomoniasis could be much improved if microscopical examination of the material were made after 1-3 hours of sedimentation. Centrifugation is equally effective. When assessing the value of an apparently negative result it must be remembered that the organism may appear and disappear over very short periods of time so that it is necessary to repeat the examination of the discharges from suspected animals. Material which cannot be examined immediately should be kept in a warm room. Some advantage may be derived from incubation at 37° C. for a few hours before the examination is carried out. The activity of the trichomonads increases appreciably when they are warmed.

Identification of the organism. For direct demonstration of the organism, purulent mucus is probably the best material. A suitable amount of vaginal discharge or of preputial washing is examined between slide and cover-slip. The outstanding characteristic of *T. fetus* is its motility, but the rounded form and sluggish movements of the organism at certain times necessitate careful observation of individual cells using the $\frac{1}{6}$ th objective of the microscope. Movement can often be increased by diluting the material with warm normal saline and, if possible, by the use of a warm slide. When movement is sluggish the undulating membrane is a characteristic feature. Identification of the moving organism should be confirmed by careful

examination of the morphological characters using the $\frac{1}{8}$ th objective.

It must be stressed that many other species of protozoa may be present in material selected for examination, particularly preputial washings.

(c) *Serological aids to diagnosis.* The technique of a blood agglutination test was described by Robertson (1941). This has proved moderately satisfactory.

The mucus agglutination test described by Pierce (1949*a* and *b*) following an observation made by Florent (1941), is, however, much more satisfactory in detecting positive cases among the infected members of a herd. Mucus samples are collected from the vagina of the suspected cow using a sterile glass tube 50 cm. long and 9 mm. diameter bent at an angle of 150° , 9 cm. from one end, and plugged with cotton wool at the other. Mucus is obtained by suction from the anterior end of the vagina. It is best collected a few days after the time of œstrus. Mucus from a case of pyometra always contains a high titre of agglutinins. Mucus from pregnant animals is unsuitable. The agglutination is recorded against the two known strains of *T. fetus*—Manley and Belfast, which are largely immunologically distinct. Suspensions of the trichomonads are prepared from cultures grown in bacto-tryptose broth on an inspissated serum slope.

The mucus agglutination test, although more satisfactory than the serum test, is nevertheless only to be interpreted on a herd basis. In an infected herd it will detect only a proportion of the positive cases.

Epidemiology

Trichomoniasis in bovines is a disease which is spread primarily by the bull. The disease in the female is self-limiting and the parasites gradually disappear. If an infected female has one calf by artificial insemination she can be returned to natural service without fear of abortion. It is not, however, absolutely certain that she may not act as a carrier of the disease.

Control

(a) *Treatment.* No form of treatment has been shown to be completely effective against *T. fetus*.

(b) *Other methods of control.*

Spread of the disease can be controlled by the use of artificial insemination using semen from a bull which is known to be free from infection. Joyner (1954) has shown that *T. fetus* can be eliminated from infected semen by storage at low temperatures in the presence of glycerol. He concludes, however, that slaughter of the infected bull remains the best way of controlling the spread of disease.

As a measure of control, it is most important that reliable breeding records should be kept. This enables the introduction of disease to be appreciated at an early stage and indicates the particular animal that has introduced the infection.

Disease is spread, it has been shown, mainly through the bull. For this reason the practice of using communal bulls should be discouraged. Artificial insemination, using adequate safeguards with the stud bulls, avoids all danger of the disease.

If an infected cow is allowed a sexual rest by not allowing it to be used for breeding until it has passed three œstral periods without the appearance of the parasite in the vaginal mucus there is a strong likelihood that it will hold to service, but freedom from infection cannot be assumed.

T. RUMINANTIUM Kust, 1936

A parasite so described has been found in the rumen of cattle and sheep and may be present in fæces contaminating the vulva of the cow. It has been suggested that *T. fetus* may have developed from *T. ruminantium* by becoming adapted to a life in the genital tract. The parasite has been described as 8-10 μ in length with three anterior free flagella, one trailing flagellum that runs along the free edge of the undulating membrane and a conspicuous axostyle.

Other species of Trichomonas

Other species have been described from various domestic animals and from man.

Trichomonads in the pig

Trichomonads can be isolated from various organs of the pig—including the nasal tract, cæcum, stomach and small

intestine. The inter-relationships of these parasites and their relationship with other species have been the subject of considerable speculation and some research. The subject is of particular importance because of evidence that species found in the pig may be transferred to cattle. They may be pathogenic and may show some cross serological reactions with the known pathogen of cattle (*T. faetus*) (Hibler, Hammond, Caskey, Johnson, Fitzgerald, 1960). In general these species do not appear to be pathogenic for pigs.

Some authorities have split the genus *Trichomonas* into *Tritrichomonas* (with 3 anterior flagella) and *Trichomonas* (with 4 anterior flagella). For the purposes of this discussion they are regarded as synonymous.

The parasites in pigs are considered by Hibler *et al.* (1960) who set out to determine the morphology and response to culture of trichomonads from the various organs of the pig and to attempt to clarify the taxonomy of the different forms. In their paper they contribute a considerable amount of useful information on isolation and culture of the organisms. Hibler *et al.* conclude that there are probably three species of trichomonads in the pig:

Tritrichomonas suis (Gruby and Delafond, 1843)

Tritrichomonas rotunda n. sp.

Tritrichomonas buttreyi n. sp.

The species were separated on the basis of morphology, distribution and behaviour in culture. The mean sizes (in μ) of the three species are given by Hibler and his colleagues as—

T. suis (parasites from three different locations in the body were measured).

$$11.42 \pm 0.51 \times 3.34 \pm 0.19$$

$$11.19 \pm 0.27 \times 3.51 \pm 0.12$$

$$11.44 \pm 0.26 \times 3.44 \pm 0.09$$

Tritrichomonas rotunda

$$8.59 \pm 0.83 \times 5.80 \pm 0.78$$

Tritrichomonas buttreyi

$$5.92 \pm 0.79 \times 3.44 \pm 0.82$$

Of these species *T. suis* has a low degree of site specificity and can be isolated from nose, stomach, cæcum or small intestine. The other two species are found in the cæcum.

T. suis shows the best survival in culture. It is very similar to *T. fætus* in cattle, is able to infect the genital tract of cattle and to cause disturbances similar to those produced by *T. fætus*. There have been some different opinions on the question of the transfer of these porcine trichomonads to bovines and the subsequent effect. Kerr (1958) reported that the vaginal mucus of a heifer experimentally infected with *T. suis* isolated from the fæces of pigs gave as positive an agglutination with "Belfast" strain *T. fætus* as with *T. suis* and he inferred that *T. suis* and *T. fætus* (Belfast) are more closely related serologically than are *T. fætus* (Belfast) and *T. fætus* (Manley).

Allsop *et al.* (1961) successfully transferred trichomonads from pig tonsillar tissue to cows. No marked pathological change was observed. Homologous mucus agglutinins were detected but only weak antibodies to *T. fætus* (both strains) antigens were observed.

PROTRICHOMONAS ANATIS Kotlan, 1923

This parasite occurs in ducks. The body is pear-shaped, 10-13 μ by 4 to 6 μ . Three anterior flagella arise from blepharoplasts. The axostyle appears to consist of two fibrils which meet at a point beyond the posterior tip of the body.

COCHLOSOMA ANATIS Kotlan, 1923

This organism has a depression on one side similar to the sucking disc of *Giardia*. The body measures 10-12 μ by 6 to 7 μ . From the anterior end arises a tuft of about six flagella which pass backwards along the surface of the body. Two other flagella arise from the anterior blepharoplasts and pass back through the body beyond the posterior extremity. Ovoid cysts with four or more nuclei are formed and are found in the fæces of infected ducks.

The parasites are found in the cæca and other parts of the gut. Their precise relationship to a disease condition remains in doubt. Campbell (1945) reported what appeared to be a serious outbreak of disease attributed to a species of *Cochlosoma* among turkey

poults in Scotland. *Cochlosoma röstratum* (Kimura, 1934) has been associated with severe mortality in turkeys in America.

HEXAMITA MELEAGRIDIS McNeil, Hinshaw
and Kofoid, 1941

This organism is associated with an infectious catarrhal enteritis in turkeys.

Morphology and life-history

The organism is bilaterally symmetrical, 6 to 12 μ in length by 2 to 5 μ wide. It is pear-shaped, with six anteriorly directed flagella and two which arise from the posterior end. The two nuclei are at the anterior end of the body. The movement of the organism is very rapid and by this character it can usually be differentiated from a trichomonad which moves more slowly and erratically. There is no evidence of the formation of a resistant cyst.

The life-cycle appears to be direct with transmission through contaminated food and water. Multiplication takes place by longitudinal division. Swezy (1915) described a type of multiple division in *Hexamita ovata* which may be the "schizogony" described by Slavin and Wilson (1953) for *H. meleagridis*.

Species affected

It is probable that infection with *Hexamita meleagridis* is confined to the turkey, the similar parasites found among other gallinaceous birds belonging to other species. One species—*H. columbæ* (Nöller and Buttgereit, 1923)—has been described as being pathogenic in the pigeon.

Symptoms

Hinshaw, McNeil and Kofoid (1938) discussed the relationship between infection with *Hexamita* and enteritis in turkey poults. Young poults up to about two months of age appear to be susceptible. Experimentally, death may result within a week of a heavy infection. Under field conditions the disease is not often associated with high mortality but there may be considerable loss of condition and severe dehydration of the tissues associated with the copious watery diarrhoea. In the later stages of the disease

the poults become listless, sit with ruffled feathers and may die seven to ten days following the first appearance of symptoms. Recovered poults harbour the parasite in the bursa of Fabricius and the cæcal glands.

Pathology

In the upper intestinal tract there is a catarrhal enteritis with marked lack of tone, watery contents and local areas of distension. There is a congestion of the glandular tissue of the cæca in the region of the ileo-cæcal openings. The liver often shows congestion.

Diagnosis

Diagnosis may be made by demonstration of the living organism in a drop of saline on a warm slide. The parasite can usually be demonstrated fairly readily in the freshly killed bird but it is practically impossible to recover the organism unless the carcase can be examined within an hour or two of death.

Control

Little is known about the epidemiology of the disease. General hygienic measures—particularly the rearing of young poults away from the adults—should be recommended.

No really satisfactory treatment has so far been devised although Almquist and Johnson (1951) found that 2-amino-5-nitrothiazole has some effect. Warm comfortable quarters for the birds assist recovery. Mangrum *et al.* (1955) used furazolidone with success at 50 mg./lb. of the food (\equiv about 0.01 per cent.).

Genus *GIARDIA*

In this genus there is a convex dorsal surface and a flattened ventral surface on which is a well-marked sucking disc.

GIARDIA LAMBLIA Stiles, 1915— Synonym, *G. intestinalis*

The organism has the typical diplozoic bilateral symmetry and is pear-shaped, varying in length from 10 μ to 20 μ ; there are two nuclei and four pairs of flagella with two axostyles (which last have been sometimes described as the axonemes) running to the posterior flagella. The organism has a very characteristic appearance in fresh preparations, swimming vigorously in an

undulating fashion by the lashing of its four pairs of flagella. Reproduction is by longitudinal binary fission.

The parasite produces characteristic cysts, oval or elliptic in shape, measuring about $9\ \mu$ to $14\ \mu$ in length with a thick wall. The encysted parasite does not fill the entire cyst there being a space at one or both ends. The two nuclei are situated at one pole of the cyst where they soon divide giving rise to 4 nuclei. The comma-shaped parabasal body also is a prominent structure.

The cyst can remain viable in moist faeces for two or three weeks. The cysts are swallowed, excystation takes place in the small intestine, trophozoites are liberated and migrate to the large intestine.

Species affected

This is a common parasite of man, in whom it has been associated with intestinal disorders. Similar organisms occur in many species of domestic animal in which their pathogenicity is in doubt. In the rabbit, examination of the small intestine may show all the glands packed with parasites, either free in the lumen of the duct or applied to the surface of the cells. The gland cells must presumably be irritated by the presence of so many organisms but there does not seem to be any tendency for the flagellates to cause ulceration or to penetrate the epithelial surface.

A species of *Giardia* is frequently found in the small gut of the chinchilla (*Chinchilla laniger*). The gut wall may be very much damaged with copious blood-stained mucus swarming with parasites. The clinical disease is characterised by an intense mucoid diarrhoea. Hagen (1950) recommends 6-9 mg. quinacrine for eliminating the parasite.

Bemrick (1961) examined faeces from dogs and cats and the small intestines from wild mice in the Minnesota (U.S.A.) area. He found about $7\frac{1}{2}$ per cent. of 2063 dogs, about 3 per cent. of 291 cats and 14 per cent. of wild mice to be infected. There was a 50 per cent. rate of infection in a colony of laboratory mice. Infection occurred markedly more frequently in young animals.

Diagnosis

Giardia can be detected by microscopic examination of the faeces. As a rule the cysts will be found, but the free flagellates sometimes occur in diarrhoeic faeces.

CHAPTER VIII

HISTOMONAS : ENTAMŒBA

THE FAMILY *MASTIGAMŒBIDÆ* CLASS MASTIGOPHORA

THE Family *Mastigamœbidæ* contains Mastigophora which have pseudopodia as well as one to three flagella. There is a single genus of veterinary importance with the species *Histomonas meleagridis*.

The *Mastigamœbidæ* can be regarded as a link with the class *Sarcodina*. Members of this class do not possess any thick pellicle and hence are capable of forming pseudopodia.

HISTOMONAS MELEAGRIDIS Smith, 1895

Histomonas meleagridis causes the disease variously known as Blackhead, infectious entero-hepatitis or typhlo-hepatitis in turkeys, domestic fowls and other gallinaceous birds (pea-fowl, pheasants, etc.).

Morphology

The causal organism is found singly or in clusters, in lesions of the cæcum and liver. The diameter of the organism varies between $8.0\ \mu$ and $15.0\ \mu$. In tissues it is amœboid but when free in the contents of the cæcum or when in culture it has a single delicate flagellum which is slightly larger than the diameter of the organism. In culture a flagellum may also be seen but it is not really characteristic ; the organism is usually sluggish and pseudopodia are much more characteristic. In occasional abnormal forms there may be more than one flagellum. When in the cæcal contents the organism can be distinguished by its characteristic rhythmical motion and it can be distinguished from a trichomonad by the absence of an undulating membrane and axostyle. In his 1919 description of the organism Tyzzer includes a number of morphologically distinct developmental stages which have not been identified by subsequent workers (Farmer, Hughes, Whiting, 1951).

The cytoplasm is often but not always granular ; there being numerous cell inclusions, cell fragments and starch granules. The nucleus is characteristic, being small, circular, usually

granular and well defined. When division is occurring an extranuclear body is apparent.

Animals susceptible to infection

Histomoniasis is essentially a disease of young turkeys and the vast majority of turkeys which contract the infection when less than a year old, die. In chickens there is considerably more resistance and, as shown by Desowitz (1951) the susceptibility of chickens decreases considerably with age.

Until comparatively recently it was rare to find disease in chickens but, apparently in association with the increase in "broiler-house" production of table-chickens there has been a considerable increase in the incidence of disease in chickens 5-10 weeks old.

A resistance to infection can be demonstrated in older turkeys as the result of previous experience of disease (Kendall, 1957).

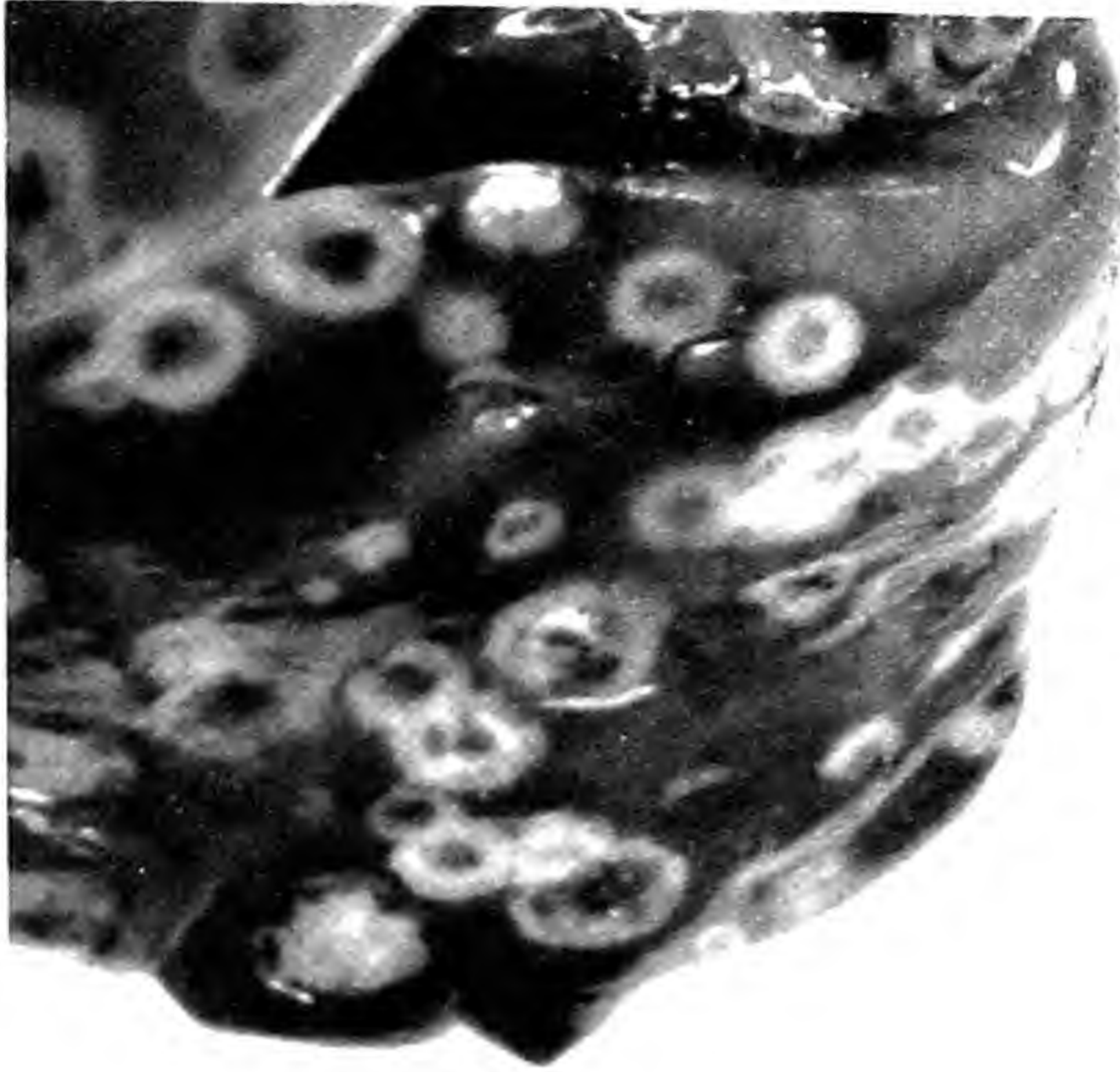
Tyzzer (1934) was able to induce an immunity in turkeys by infecting them, by rectal injection, with avirulent strains of *Histomonas meleagridis*. Mild, non-progressive lesions were demonstrated in the cæcal mucosa of birds which were shown to be resistant to subsequent infection with virulent strains.

Lund (1959) has apparently demonstrated the existence of avirulent strains and that they can elicit some degree of resistance to further infection with virulent strains.

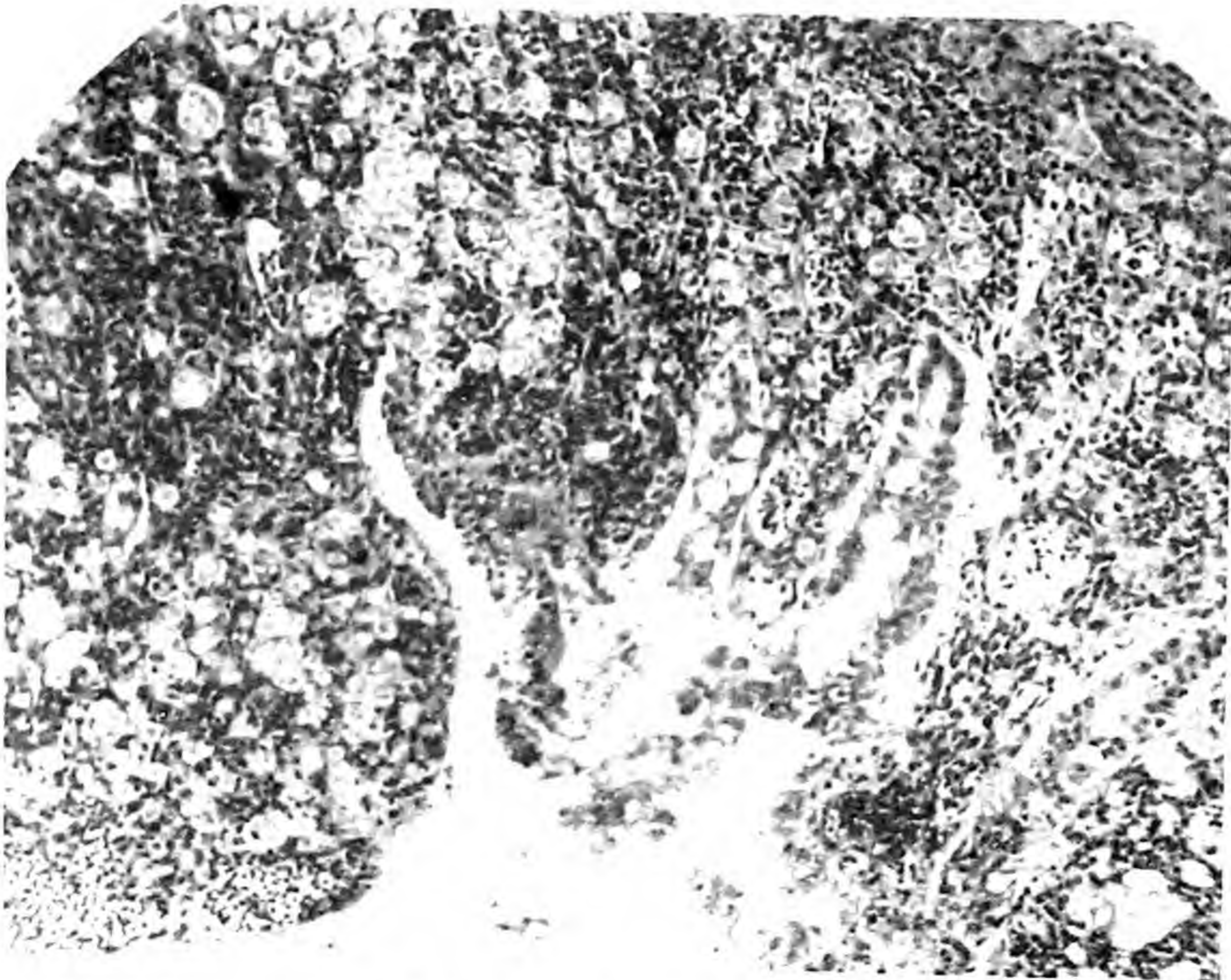
Life-history and transmission

Reproduction of the parasite is by binary fission. Primary infection appears to occur in the cæcum. Later involvement of the liver is characteristic of the disease in the turkey but not in chickens, although in young birds it is sometimes seen. Live histomonads may be present in the fæcal discharges but there is no resistant cystic stage and outside the body the parasite is very susceptible to destruction by adverse conditions such as low temperatures and desiccation. Tyzzer (1934) was able to demonstrate direct transmission from bird to bird, but it seems improbable that this occurs very commonly under average field conditions. There is little doubt that the main route of infection is via the embryonated egg of the nematode *Heterakis gallinæ* which commonly occurs in the cæca of poultry.

FIG. 15(a)

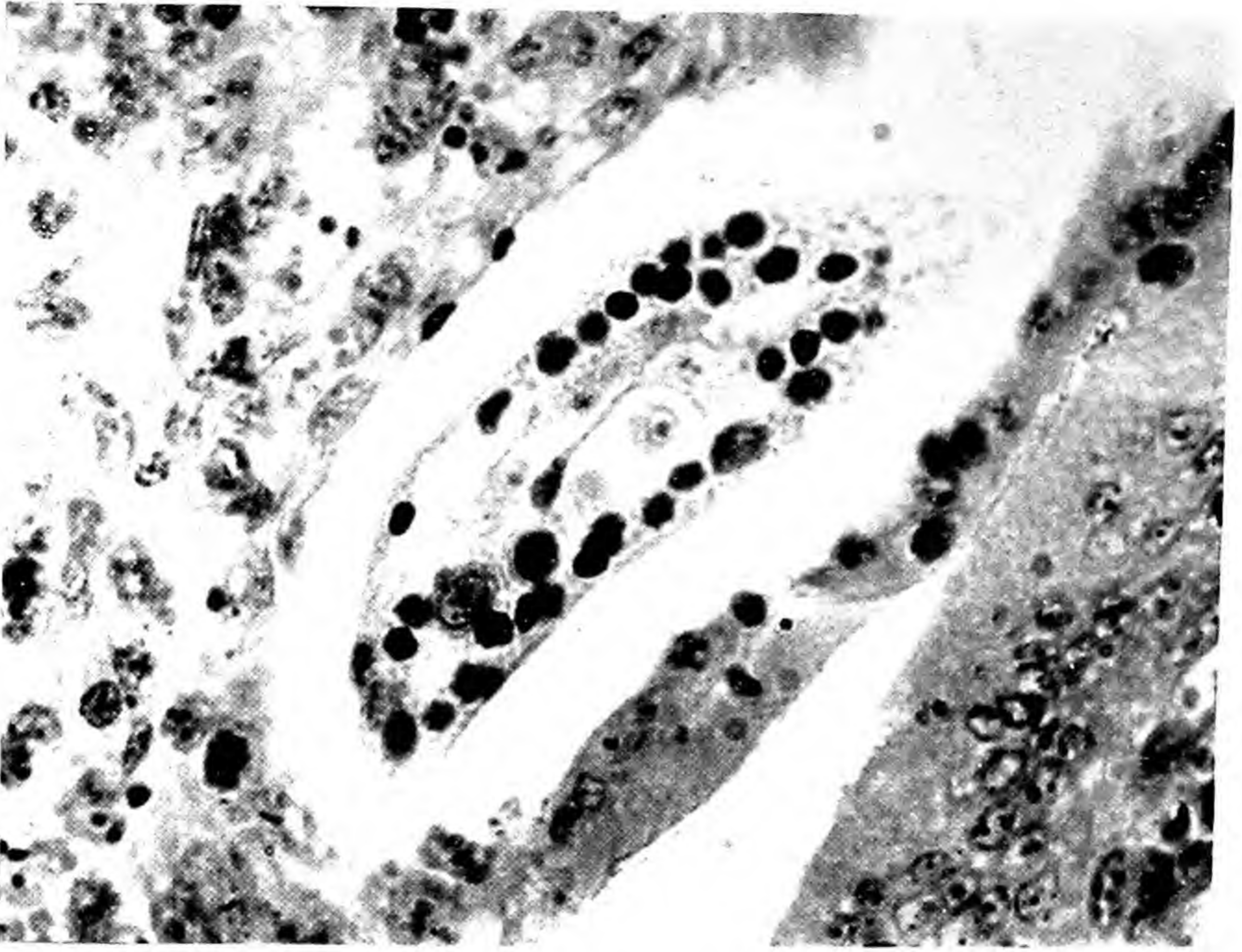


Liver of turkey showing lesions caused by *Histomonas meleagridis*

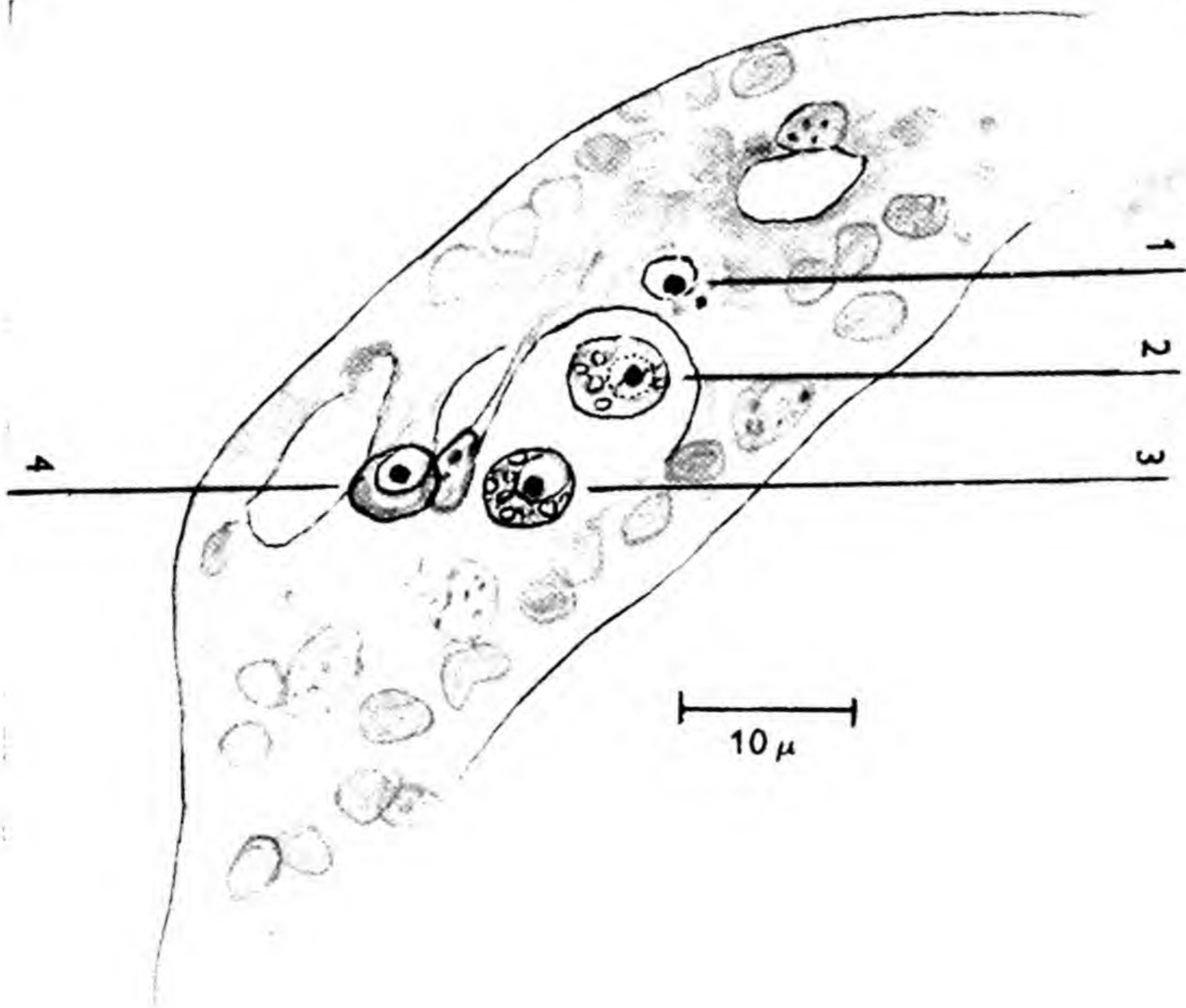


Caecal tissue heavily infected with *Histomonas meleagridis*

FIG. 15(b)



A photomicrograph of a larva of *Heterakis gallinæ* containing *Histomonas meleagridis*



The association between *Histomonas meleagridis* and *Heterakis gallinæ* was first demonstrated by Graybill and Smith (1920) who produced histomoniasis in young turkeys by feeding them with embryonated eggs of the nematode. Subsequent observations have indicated that the parasite is carried within the egg. It is thus able to withstand a considerable degree of desiccation and to remain viable for many months. It has not so far proved possible to demonstrate the parasite in the egg.

Kendall (1959) has illustrated what he believes to be *Histomonas meleagridis* in a young larva of *Heterakis gallinæ*.

Passage of the parasite in blood from an infected bird has been demonstrated by McGuire and Morehouse (1958).

Clinical Symptoms

Clinical symptoms of the condition appear not less than eight days after infection and often considerably later. Depression, drooping wings, ruffled feathers, lowered head and most characteristic of all, sulphur-yellow droppings, are the main symptoms of infection in the turkey. In the domestic chicken yellow droppings seldom occur and there may, in fact, be no really diagnostic signs of disease.

The disease is most acute in young poults which may die within 24 hours of first showing symptoms. The name "Black-head" is really a misnomer, the head being no darker than in most other disease conditions of the turkey.

Pathology

Usually the lesions are confined to the cæca and the liver. One or both cæca may be involved. The earliest lesions in the cæcum are small raised pin-point ulcers in which the organisms may readily be demonstrated. Later the lesions become much enlarged and thickened with yellowish patches scattered along the surface of the serosa. Occasionally these ulcers may perforate the cæcal wall and result either in a fatal peritonitis or in the development of adhesions to the other organs. When opened, the cæcum is found to contain a hard caseous core to which the lining epithelium of the cæcum adheres. The histomonads are believed to reach the liver via the blood stream. The resulting hepatic lesions usually have a characteristic circular depressed appearance with

yellowish necrotic centres and a greyish peripheral region with radiating streaks of necrosis. Sometimes, particularly in young turkeys and in chickens, the lesions may be predominantly greyish-white and more diffuse in appearance. In any event the lesions extend deeply into the liver parenchyma. The healing processes which sometimes occur in both liver and cæcum are marked by the development of fibrinous and lymphoid tissue. In recovered turkeys the extensive scarring which results may remain for a very long time.

The pathology of experimentally induced histomoniasis is described by Malewitz *et al.* (1958).

Typical gross lesions were seen in cæca, liver, spleen and kidneys. The lesions were characterised by hyperæmia, hæmorrhage, lymphocytic infiltration, macrophages, multi-nucleated giant cells, necrosis and usually a serous exudate. There was a granulomatous type of inflammation accompanied by necrosis. In the lungs, pancreas and heart there were areas of hyperæmia and serocellular exudate but parasites were not demonstrated.

Diagnosis

Diagnosis is made on the evidence of yellow droppings and on the post-mortem appearance.

Epidemiology

Heterakis gallinæ is well known as a common parasite of the domestic fowl and experimental evidence suggests that nearly all *Heterakis* carry the Blackhead organism. Many, if not all, domestic fowls presumably carry symptomless infection with *Histomonas*. Acute outbreaks of histomoniasis are likely therefore to arise when young turkeys are associated with domestic fowls or when young turkeys are placed on ground recently vacated by other poultry. Outbreaks of disease vary very much in intensity but it can safely be assumed that the incidence and severity of disease on a particular piece of ground occupied by poultry will increase as the years go by.

Control of the disease

(a) *Husbandry*. It is clear from what has been said that the methods of control are essentially those which avoid infection

with *Heterakis*. Regular dosing of the flock with phenothiazine may help to reduce the incidence of nematode infestation. It is essential to avoid contact between turkeys and domestic fowls, including the use of broody hens for rearing poults, and to use clean land for rearing—preferably short turf which has not previously been used for poultry. Wire floors which raise the birds above contact with the ground are now used by many successful turkey farmers.

(b) *Therapy*. The literature on the chemotherapy of histomoniasis has been reviewed by Wehr *et al.* (1958).

In the past various arsenical compounds have been recommended for use both prophylactically and curatively. Of these Stovarsol (sodium acetarsol) has some value if treatment is commenced before the time of infection. In 1950 Waletzky, Clark and Marsen introduced 2-amino-5-nitrothiazole. Both this compound and the related acetamido derivative proved very effective both for control of established disease and, at lower concentrations, for prevention. 2-amino-5-nitrothiazole can be given in food or in water. It is recommended for use (Joyner and Kendall, 1955) at a concentration of 0.05 per cent. of the food as a preventive or at 0.1 per cent. of the food for curative purposes. If used for the control of the established disease 14 days' treatment at the 0.1 per cent. level must be followed by continuous treatment using 0.05 per cent. of the drug for at least 2 months. Relapse, which is characteristic of most if not all therapeutic substances used for the control of histomoniasis when therapy is commenced after the time of infection, may occur even when therapy is continued for as long as 35 days at the 0.1 level of drug.

2-acetamido-5-nitrothiazole is less soluble than is 2-amino-5-nitrothiazole so that its use is confined to capsule or to admixture with the food. It can be given at approximately half the concentration of 2-amino-5-nitrothiazole to produce about the same therapeutic effect.

Furazolidone at concentrations of 0.01 per cent. to 0.02 per cent. also is effective as long as medication precedes infection.

Of newer drugs both "Nithiazide" and "Histostat" (4-nitrophenyl-arsonic acid) are reported as being effective.

Culture

Histomonas meleagridis can be maintained in culture using methods described by Drbohlav (1924) and other more recent workers, e.g. De Volt (1943). Certain bacteria are ordinarily essential for the growth of the organism in culture although Lesser (1961) has recently described the culture of *H. meleagridis* in a modified tissue culture medium without the presence of live bacteria. Rice starch is ingested. Both flagellate and amœboid forms of the parasite may be observed in culture.

The use of such cultures in experimental investigations involving the laboratory infection of turkeys or chickens is not common mainly because a very large volume of culture would be required. In addition there is evidence that laboratory strains of *Histomonas* tend to lose virulence.

Experimental investigations

Clinical histomoniasis, which does not appear to differ from the field disease, can be induced in the laboratory by feeding embryonated *Heterakis* eggs of which at least 1000 per bird should be used. All *Heterakis gallinæ* eggs, in Britain at least, are likely to carry the infection. Alternatively, an active infection may be induced, with an incubation period about three days shorter than that induced by *Heterakis* eggs, by the rectal injection of a fresh warm suspension of mashed liver or (preferably) cæcum from a clinical case of histomoniasis. The suspension *must* be kept warm and used within two hours of preparation.

The genus *ENTAMŒBA* class Sarcodina

The genus *Entamœba* is distinguished from other members of *Sarcodina* by the possession of a vesicular nucleus with a comparatively small *karyosome* (a conspicuous granule), near the centre, and with peripheral chromatic granules attached to the nuclear membrane. There is chromatin in the *karyosome* and in the peri-karyosomal region.

Two other related genera, *Endolimax* and *Iodamœba*, occur in domestic animals and are distinguished by details of the structure of the nucleus and the morphology of the cysts.

The genus *Endolimax* contains non-pathogenic amœbæ which

are small and in which the nucleus has a large irregular excentric karyosome and no peripheral chromatin. Mature cysts are usually oval and 4-nucleate. Species of *Iodamoeba* are similarly non-pathogenic. The nucleus has a large spherical karyosome surrounded by achromatic granules ; mature cysts are of irregular shape with one nucleus and abundant glycogen.

The group is reviewed by Hoare (1959) who records species of amœbæ of one or more genera in domestic animals, monkeys, rodents and domestic poultry. Usually infection is benign. *Entamoebæ* with 8-nucleate cysts (e.g. *E. coli*) are separated from those with 4-nucleate cysts (e.g. *E. histolytica*). This is an important distinction because *E. histolytica* (4 nuclei) is an important pathogen of man and may occur in various other species. *E. coli* (8-nuclei) is a benign parasite living in the large bowel.

E. HISTOLYTICA Schaudinn, 1903

This parasite causes amœbic dysentery in man. Occasional outbreaks associated with a morphologically similar parasite have been described in numerous wild and domestic animals. (See Kudo, 1954 and Hoare, 1959).

Morphology

The trophozoite is an active parasite measuring 9-20 μ or more, in diameter. The cytoplasm is well differentiated. Large lobopodia, developed from ectoplasm, are formed. Food vacuoles contain erythrocytes, tissue cell fragments and leucocytes and the presence of these is one of the clearest indications that the species is pathogenic. In stained material it can be seen that the nucleus is bounded by a peripheral membrane, that it contains small peripheral granules and that the small karyosome is centrally placed.

Distribution

In man the parasites live in the lumen and in the wall of the colon. Through the portal vein parasites may invade the liver and other organs.

Pathogenicity

In man the parasite may produce the disease of amœbic dysentery, characterised by ulceration of the colon and liver.

Necrosis of the epithelial cells occurs and the intra-glandular tissue becomes involved. The ducts of the gland become blocked, abscesses are formed and ulceration results.

In animal hosts, amœbæ produce lesions of the stomach, duodenum, ileum, colon and liver.

Hoare (1959) quotes an account of an outbreak in *cattle* of disease with dysentery, apparently associated with an epidemic of amœbiasis among Africans. *E. histolytica* has been recovered also from the lungs of zebu cattle in Dakar.

In *dogs* Hoare records four intestinal species—*Entamœba coli*, *E. histolytica*, *E. caudata* and *Endolimax nana*.

Monkeys may harbour amœbæ apparently indistinguishable from those in man—usually without displaying symptoms of disease.

In *dogs*, *E. histolytica* may occur throughout the large gut with extensive erosion of the mucosa and with deep ulceration. Abscesses may occur in the liver as in man. There is a variable clinical course—acute disease may ensue with a fatal termination or there may be a mild infection with spontaneous recovery. *Dogs* may become carriers. Epizootics have been described.

Rats are susceptible to experimental infection with human strains of *E. histolytica* and can be used for testing amœbicidal drugs.

Life-History

In the colon of man the trophozoites multiply by binary fission, the tissue-penetrating forms actively forming pseudopodia. For a time the parasites remain small after division. They are sluggish and are known as the pre-cystic forms. These smaller forms encyst, the cysts being $7\ \mu$ to $20\ \mu$ in diameter and appearing as greenish refractile spherical bodies. The cyst wall is $0.5\ \mu$ thick but is colourless and transparent. Within the cyst the nucleus of the organism divides to form four nuclei. The mature cyst contains diffused glycogen and elongate refractile rod-like bodies with rounded extremities which stain deeply with hæmatoxylin or iodine. These are the chromatoid rods.

The chromatoid rods vary in length from $5\ \mu$ to $10\ \mu$ and may sometimes be filamentous in form or irregular. There are usually three or four in each cyst. They tend to be absorbed and to

disappear as the cyst matures. No further changes take place in the cyst until it is ingested by a fresh host. The process of excystation has been observed *in vitro*, a single tetranucleate organism emerging through a minute pore in the cyst wall. The tetranucleate amœba produces a new generation of trophozoites consisting of eight uninucleate individuals which grow into normal sized amœbæ. No evidence of sexual reproduction has been noted.

Diagnosis

The disease is ordinarily diagnosed by the examination of diarrhœtic fæces for the presence of trophozoites and the examination of formed fæces for cysts. It is necessary to differentiate the pathogenic *E. histolytica* in man from the non-pathogenic *Entamœba coli*.

Entamœba coli (Grassi, 1879)

This species is larger than *E. histolytica*, the pre-cystic forms being $20\ \mu$ to $30\ \mu$ in diameter. It is not so active as *E. histolytica* and does not ingest red blood corpuscles. The ectoplasm and endoplasm are not so distinctly differentiated and the nucleus is more clearly visible in fresh preparations. The cysts are larger than those of *E. histolytica* being $10\ \mu$ to $30\ \mu$ in diameter. They rarely contain chromatoid rods and characteristically there are eight nuclei instead of only four.

Treatment of *E. histolytica*

In man, emetine hydrochloride is given by subcutaneous or deep intramuscular injection daily for about ten days. The drug is toxic and a prolonged course may induce drug fastness. Emetine-bismuth-iodide may be given in hard gelatine capsules. Quinoxyl (Chinioform, Yatren) is sometimes used as a retention enema.

Emetine injections are a specific against amœbic hepatitis. Successful treatment of the disease in dogs is recorded by Ware (1916) giving 0.5 to 1.0 gm. emetine hydrochloride. Boyd (1931) found that emetine-bismuth-iodide was toxic for dogs and recommends acetarsol (stovarsol).

CHAPTER IX

THE CLASS SPOROZOA

HEPATOZOOM-CRYPTOSPORIDIUM-EIMERIA-ISOSPORA

MEMBERS of the class *Sporozoa* are, without exception, parasitic. They are characterised by a particular type of multiple division (*schizogony*) and by the production of resistant spores. The trophozoite is not usually actively motile and it possesses no special organs such as cilia or flagella.

The Family *Eimeriidae* contains parasites which have life histories typical of the class. *Macrogametocytes* and *microgametocytes* develop to form (single) *macrogametes* and numerous *microgametes*. The dissimilar gametes fuse to form zygotes. The resistant *oocysts*, when first passed with the fæces of the host, are usually immature.

Parasites belonging to the Family *Hæmogregarinidae* have two hosts. They are found in the circulatory system of vertebrates and in the digestive system of invertebrates. One genus, *Hepatozoon*, is of some veterinary interest.

THE FAMILY HÆMOGREGARINIDÆ

HEPATOZOOM MURIS (Balfour, 1905)

This parasite occurs in rats and mice. Schizogony occurs in the cells of the liver. Syngamy and sporogony occur in mites. Rats and mice are infected by ingesting mites.

HEPATOZOOM CANIS (James, 1905)

Life-history

Schizogony occurs in the endothelial cells of the spleen, bone marrow and liver of dogs, where the schizonts appear as round or oval bodies with 30-40 nuclei, almost filling the invaded cells. Gametocytes invade leucocytes and are seen as bodies about 6 μ by 3 μ with a nucleus and cytoplasm containing pink granules. They are surrounded by a delicate capsule. In citrated blood

the parasites can be seen to leave the leucocyte and emerge from the capsule. Syngamy and sporogony occur in the tick, *Rhipicephalus sanguineus*, in which sporocysts develop. Infection of dogs is believed to occur by ingestion of ticks. There is an account of infection in the dog by Rau (1925).

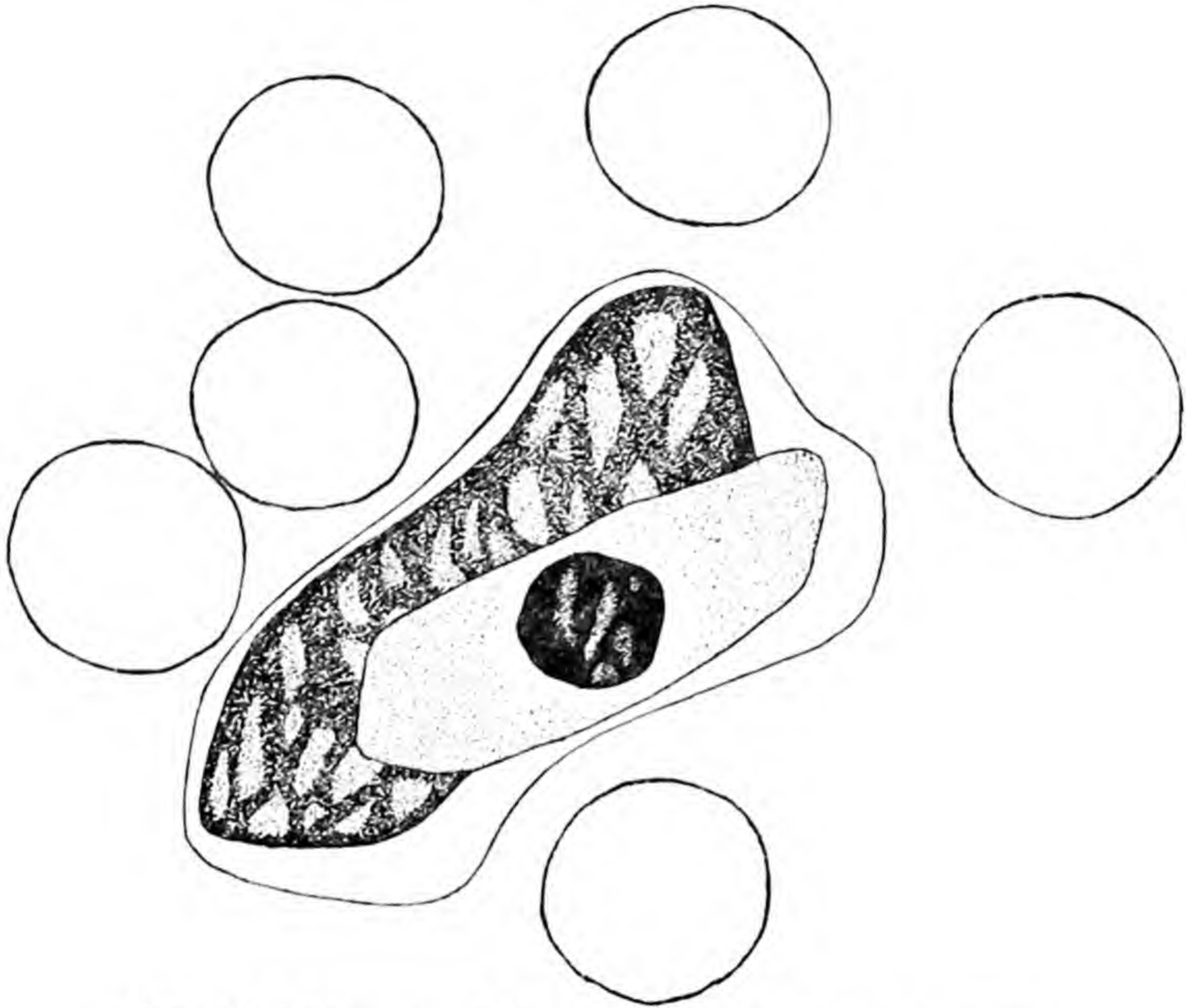


FIG. 16.—Gamete of *Hepatozoon canis* in blood smear.

Pathogenicity

The parasite, which occurs in Asia and Africa, has been identified as being pathogenic to dogs, causing irregular fever, anæmia and enlargement of the spleen, with death in four to eight weeks. *Hepatozoon* is, however, commonly encountered in apparently healthy dogs.

H. ROTUNDA Patton, 1910

This is a more spherical species of *Hepatozoon* which was first recorded in jackals, but was later found to occur also in dogs in the Sudan and West Africa (Leger, 1912).

THE FAMILY EIMERIIDÆ

The Family is divided into a number of genera of which four are of veterinary interest. These are :—

(1) *Cryptosporidium* ; characterised by the formation of an oocyst which when mature contains four sporozoites but no sporocysts.

(2) *Isospora* in which the ripe oocyst contains two sporocysts each of which contains four sporozoites.

(3) *Eimeria* in which the ripe oocyst contains four sporocysts each of which contains two sporozoites.

(4) *Tyzzeria*—in which the ripe oocyst contains eight free sporozoites.

Of these, *Cryptosporidium* includes small parasites found exclusively on the surface of mucous membranes. They are seldom associated with disease in domestic animals but must be differentiated from developmental forms of the other coccidia.

The *Isospora* includes a group of parasites which are found principally in carnivores. Their pathogenicity is not very great, as a rule, and the group is of small practical importance by comparison with the members of the genus *Eimeria*.

The great majority of the parasites known as *coccidia* belong to the genus *Eimeria*.

The general account of the coccidia which follows applies particularly to *Eimeria*, but with some modification to the other members of the Family.

THE COCCIDIA

Geographical distribution and incidence of infection

Coccidia appear to be ubiquitous in distribution and there is reason to believe that the same species act as the principal pathogens throughout the world. For example, the well-known disease of domestic fowls, *cæcal coccidiosis*, is everywhere caused by the coccidium *Eimeria tenella*. The incidence of infection is, moreover, exceedingly high. In Great Britain, infection with coccidia can be demonstrated in about 90 per cent. of fowls and rabbits. The incidence in cattle, sheep and pigs is unknown, although infection is certainly not rare, but the voluminous fæces make detection more difficult. It must, however, be stressed at the outset that many species of coccidia are of low pathogenicity

and that infection even with the known pathogens does not necessarily produce clinical disease.

Habitat

Coccidia, particularly those of the genus *Eimeria*, appear to be very strictly host-specific, even closely related host species carrying their own particular sorts of parasite. Coccidia of sheep and goats are usually classified together but this is probably in the main an index of the very limited knowledge of the group. In addition to being host-specific the coccidia are commonly organ-specific. Thus *Eimeria tenella* is confined to the cæcum of the chick while *E. necatrix* spends the first part of its development in the small intestine but later migrates to the cæcum. *E. stiedæ* is found only in the liver of the rabbit, while *E. truncata* parasitises the kidney of the goose. The very great majority of the coccidia of veterinary interest are, however, parasites of the epithelium lining the intestinal tract.

Some exceedingly interesting observations made by Davis, Boughton and Bowman (1955) and Davis, Bowman and Boughton (1957) have shown that *E. alabamensis* can develop within the nucleus of cells in cattle. Schizonts, sexual stages and oocysts can all develop within the nuclear membrane of cells in the epithelium of the small intestine.

Life-history and reproduction

At the commencement of the asexual part of the life-cycle the coccidium is called a *trophozoite* and is found living within a tissue cell of the host. Growth of the trophozoite, which finally fills the cell, leads to the formation of a *schizont* which reproduces by multiple division. In the process of schizogony the nucleus divides into a number of parts, each of which acquires a small portion of cytoplasm. The resulting motile fusiform *merozoites* escape from the host cell and penetrate other cells, becoming *second generation schizonts* by growth. This process of schizogony may be repeated for several generations. The different generations of schizonts may sometimes be distinguished morphologically and physiologically. They may, for instance, parasitise different parts of the mucosa and they may react differently to chemotherapy. Eventually, possibly as a response to the developing

immunological response of the host, some of the merozoites become differentiated into male and female forms and the sexual part of the cycle is initiated.

Immature sexual forms are known as *gametocytes*, the male being known as the *microgametocyte* and the female as the *macrogametocyte*. The microgametocyte divides to form a large number

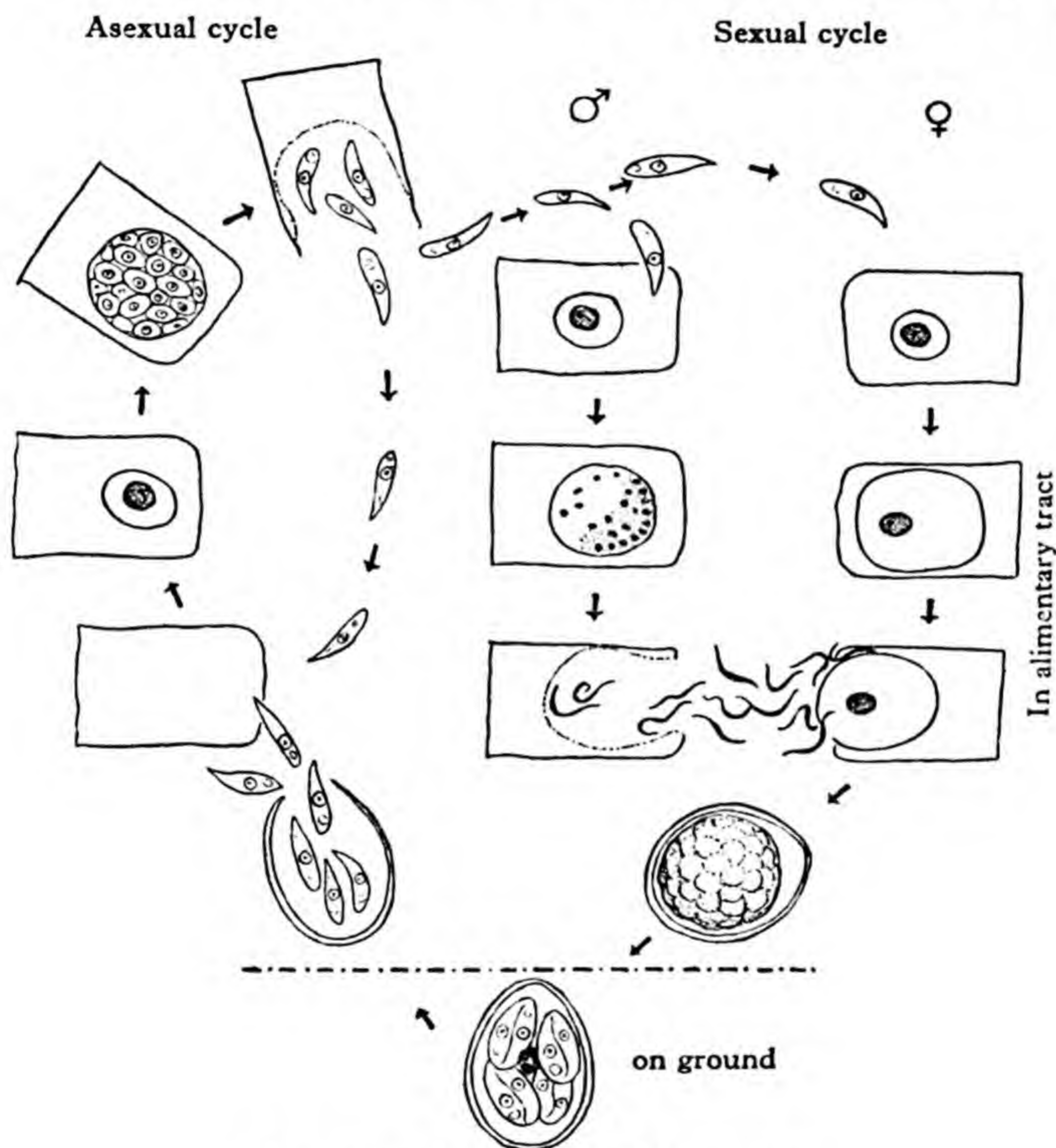


FIG. 17.—Life-cycle of *Eimeria*.

of motile *microgametes*, while development of the macrogametocyte leads to the production of a single *macrogamete*. Fertilisation of the macrogamete by a microgamete results in the production of a *zygote* which secretes a cyst wall and becomes an *oocyst*. The oocyst passes out of the host and matures on the ground to form sporoblasts which in turn divide to form *sporocysts*.

In the genus *Eimeria* the mature oocyst, when ready to infect a new host, contains four sporocysts each of which contains two *sporozoites*. It will be remembered that the mature oocyst of the

genus *Isospora* contains two sporocysts each of which contains four sporozoites.

Infection of the new host ordinarily occurs through ingestion of the intact oocyst, but it has been shown that sporozoites remain viable within the sporocyst for at least a limited period after fracture of the oocyst. In the laboratory, excystation of the sporozoites may be observed on a warm slide in the presence of 5 per cent. alkaline trypsin, following preliminary fracture of the oocyst wall as described by Goodrich (1944).

It was originally believed that infection of the host cells occurred through a direct invasion by sporozoites. Recent observations have shown, however, that in some species at least (e.g. *E. necatrix* (Van Doorninck and Becker, 1957)) the sporozoites first invade the epithelium at the tips of the villi of the small intestine but are then transported in macrophages through the *lamina propria* of the villi to reach the epithelium at the depth of the intestinal glands. Here further development of the trophozoite (as it now is) occurs. Sporozoites of *E. tenella* can similarly be transported by macrophages (Challey and Burns, 1959).

Pathogenesis, Pathology and Symptomatology

Under conditions of extensive husbandry the coccidia of domestic animals are relatively benign parasites. All domestic animals are likely to be infected for at least a part of their lives. Disease arises as the result of a rate of infection which is heavy in relation to the previous experience of the host and which is greater than the rate at which resistance develops. As will be shown later, the development of a specific resistance to reinfection is one of the most important aspects of the epidemiology of coccidiosis. The pathology and symptomatology vary, therefore, very greatly according to the species of coccidium concerned and to the degree of resistance which the particular host has acquired. Major pathological changes are usually due to the development of schizonts, particularly if they are sited, as are those of *Eimeria tenella*, in the deeper layers of the intestinal mucosa. In such a case the sole cause of death appears to be the excessive hæmorrhage which is associated with the simultaneous maturation of the second generation schizonts. In other instances, e.g. *E.*

stiedæ, blockage of the bile ducts leads to liver disfunction which is caused partly by the development of the schizonts, but, probably to a larger extent, by oocyst production. Heavy infection with *E. tenella*, with characteristic violent hæmorrhage, causes acute disease but coccidiosis is frequently far more chronic in character. The disease may pass through an initial acute phase the most characteristic symptom being diarrhœa, often with the discharge of large amounts of mucus and occasionally a little blood. During

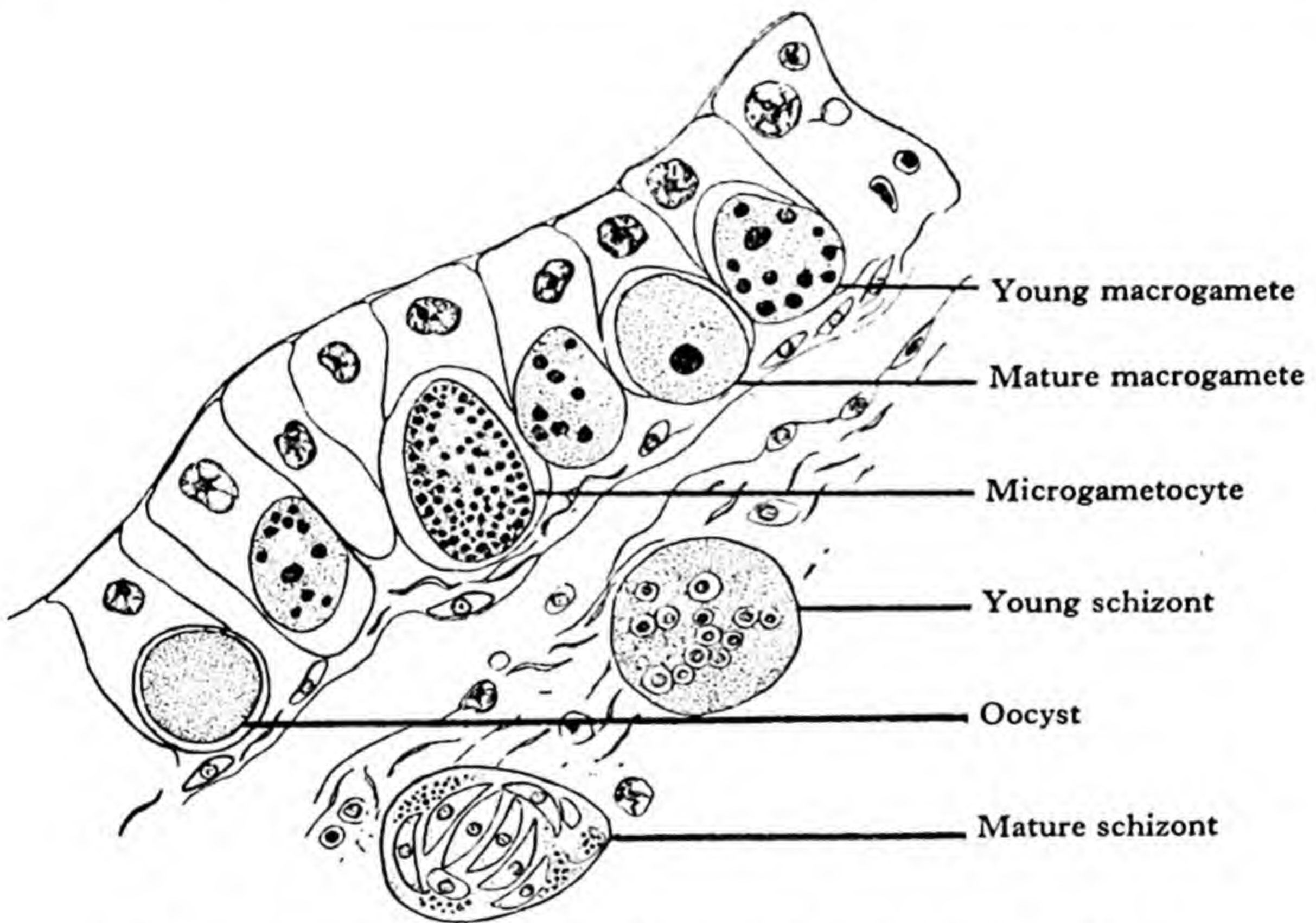


FIG. 18.—*E. tenella* in cæcal mucous membrane of chick.

this time inappetence is usually apparent although the patient will continue to drink. There is likely to be a rapid loss of flesh and some anæmia. Later some degree of recovery is evident but, depending on the extent to which the mucous membrane has been damaged, there will be impairment of function. In many instances, this secondary phase is economically of the highest importance, for it results in the production of chronically unthrifty stock which may never recover full productivity.

Immunity

It has already been indicated that the development of specific resistance to reinfection is characteristic of coccidiosis. The

problem has best been studied with *E. tenella*. In the chicken, resistance to further infection with this parasite appears to develop within a few hours of the time when the schizonts begin to develop and by about the fifth day following initial infection with a few thousand oocysts, chicks are usually able to survive all but the heaviest challenge. The resistance so acquired appears to be quantitatively related to the size of the initial dose of oocysts.

The nature of the resistance to reinfection is still in doubt. Work reported by Ripson, Johnson and Herrick (1949) suggested that the resistance was local in origin and might have a physiological basis. There is other evidence tending to confirm this view. There is, however, a growing volume of evidence (Burns, 1958 ; Burns and Challey, 1959 ; McDermott and Stauber, 1954 ; and Rose, 1959) which suggests that infection elicits the formation of circulating antibodies and that these antibodies may have an effect on some developmental stages of the parasites.

Many species of coccidium seem to have short life histories and all development of the parasite ceases within two or three weeks of the time of infection. Oocysts may, however, be retained mechanically within the host for much longer periods of time. With other species, the period of time over which generations of schizonts and oocysts can be produced may be extended considerably. There is, however, little or no precise information on the length of time for which an infection may be retained without reinforcement, owing to the almost insuperable difficulties which attend the rigid isolation of experimental animals.

Epidemiology

The key to the epidemiology of the disease lies in the way in which resistance is acquired as the result of initial light infection, the excessively large numbers of infective oocysts which can accumulate under certain environmental circumstances and the high susceptibility of previously uninfected stock to the pathogenic effects of the disease. In general, adult stock will be resistant to the disease ; young stock will be susceptible. Disease can be avoided under conditions of extensive husbandry where the rate of infection is low, while under intensive conditions the rate of infection is likely to be high. Disease will be most apparent under conditions where groups of young susceptible stock follow

one another on the same piece of ground, or through the same pens. Large amounts of infective material are excreted by infected susceptible stock but the maturation of the oocysts on the ground may be delayed by environmental conditions. The sporulation of the oocysts of coccidia is assisted by warmth and moisture. It may be totally inhibited by dryness and by low temperatures or lack of oxygen. Hence there is often a close correlation between climatic conditions and outbreaks of coccidiosis. Oocysts may remain viable for some weeks when unsporulated and for months when they have become sporulated.

So far there is little evidence that susceptibility to infection is affected by anything except natural (species) resistance or by previous infection. There is some evidence from the field that outbreaks of coccidiosis in cattle frequently follow inoculation campaigns against rinderpest, the inference being that the inoculation has some effect on the normal resistance of the cattle. It seems far more reasonable, however, to believe that such epidemics follow the unusual congregation together of large numbers of animals from different areas and the bringing together of susceptible and infected stock.

In the laboratory, there is so far little evidence that any environmental factors affect the specific resistance of the animal although such factors may affect the degree of morbidity of the disease. Chicks, for example, appear to be uniformly susceptible to infection with cæcal coccidiosis but there may be considerable differences in the rates of mortality according to the temperature of the environment at the time hæmorrhage occurs.

There is evidence, too, in this instance, where hæmorrhage is the primary cause of death, that vitamin K levels may affect the rate of mortality (Tugwell, *et al.* 1957).

Diagnosis

Diagnosis is ordinarily carried out by the direct examination of smears of intestinal contents and of fæcal samples, using a salt flotation technique. The oocysts of coccidia are identified by morphological characters which may be summarised as

- (1) Length and breadth
- (2) Shape and colour

- (3) Presence or absence of a micropyle
- (4) Thickness of the wall and presence or absence of residual bodies and refractile granules.

Identification of the parasite

Ordinarily the size and general appearance of the oocysts should be sufficient for their identification by reference to a

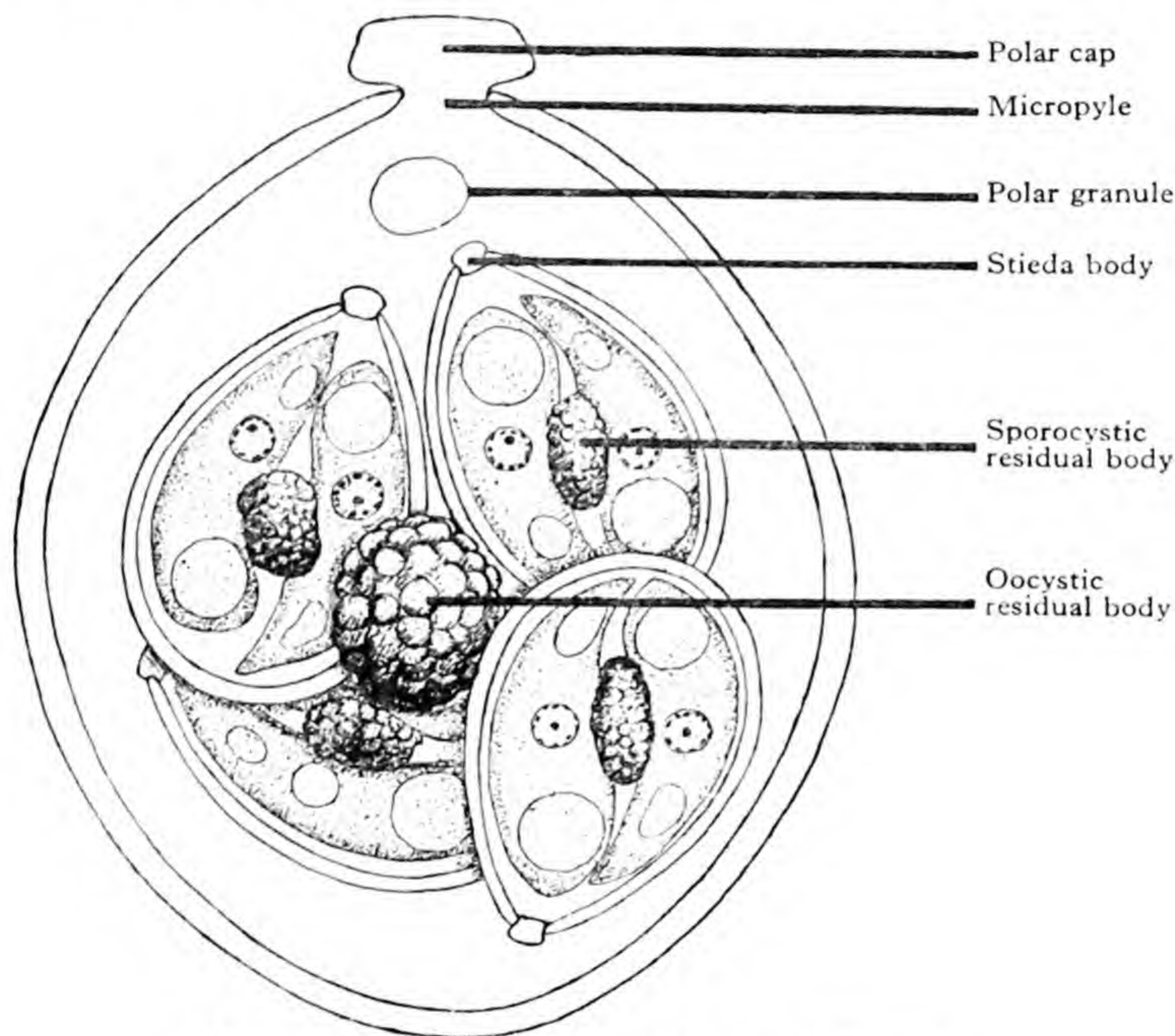


FIG. 19.—Characters of an oocyst of *Eimeria*.

check list of the species known to parasitise the particular host species, but recent work on the day to day variation in size of oocysts even from a single bird has shown that in several instances size alone is of limited value as a criterion of species. Laboratories which specialise in such work may need to confirm diagnosis by observation of the sporulation rate of oocysts (kept under absolutely standard conditions), by inoculation of susceptible hosts, by observation of the prepatent period (the period after infection when oocysts first appear) and by examination of the parts of the host tissue which are preferentially parasitised.

The characters of a sporulated oocyst of *Eimeria*

The oocyst wall possesses an outer and inner membrane and under the microscope has a double contour. The shape of the oocyst may be spherical, ellipsoidal, ovoidal or cylindrical. Some oocysts, even when fully developed, show a visible micropyle and through this the inner membrane may project to form what is known as a polar cap. A clear refractile granule occurs, and is usually seen near the micropyle. It has been named the polar

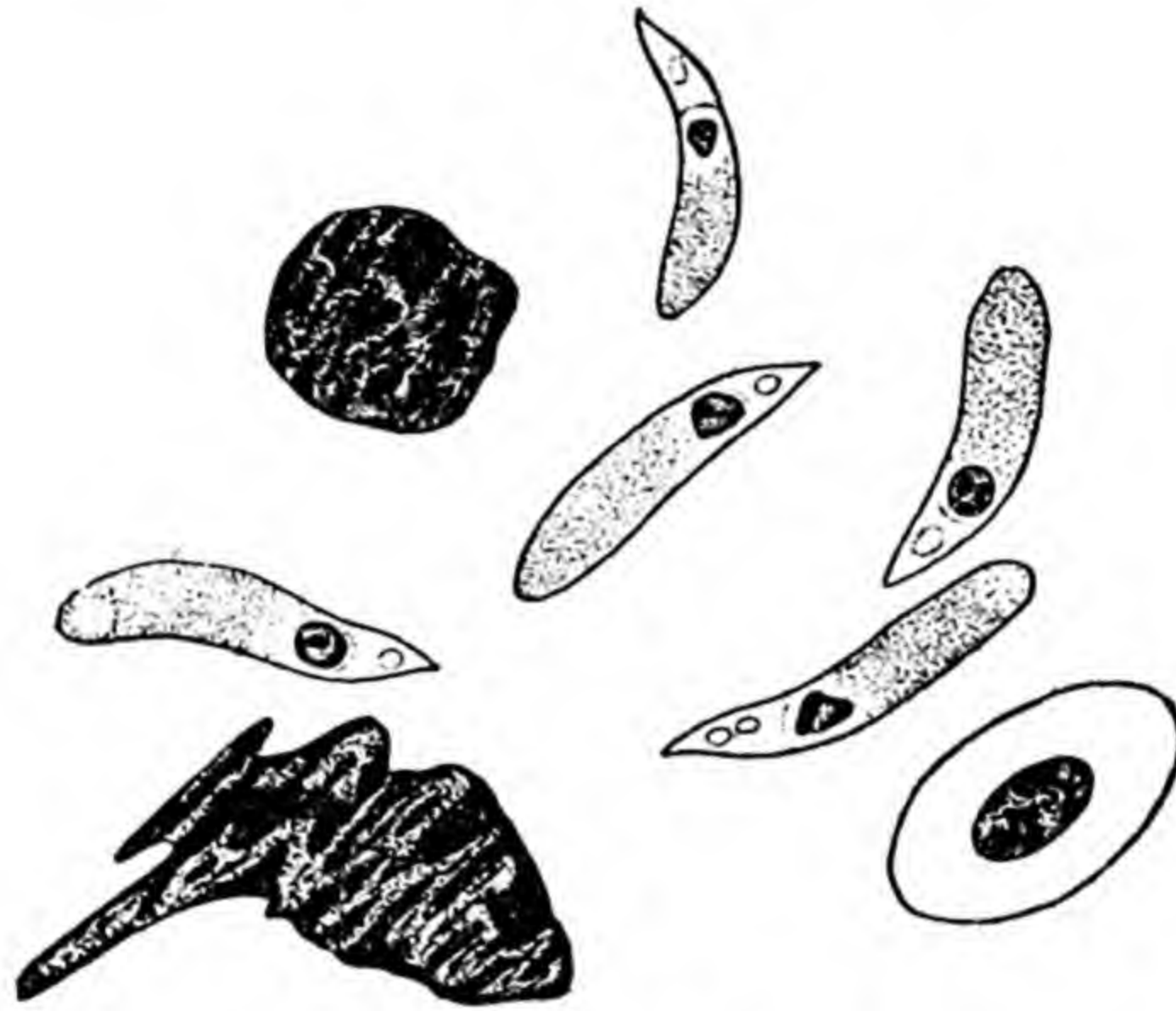


FIG. 20.—Merozoites of *Eimeria tenella* from scraping of cæcal mucous membrane of fowl with red blood corpuscle and leucocytic debris.

granule, though it is not always polar in position. Within the oocysts are seen the four sporocysts and the oocystic residual body, if this occurs. Within the sporocysts the two banana-shaped sporozoites, with their nuclei, may be seen, and the sporocystic residual body, if present. At one end of the sporocyst may be another refractile granule, the *Stieda body*. There are also large refractile granules at the broader end of the sporozoites, and smaller granules at the more pointed end.

The morphology of Merozoites

Merozoites are elongated organisms, varying in size and shape but usually about $6\ \mu$ long by 2 to $4\ \mu$ broad. One end is pointed and clear, and the other rounded, the cytoplasm being loaded with granules. The nucleus is nearly central and is typically vesicular with a large central karyosome.

Diagnosis of disease

A diagnosis of disease, caused by infection with coccidia, can rarely if ever be made solely on the basis of the recovery of oocysts from faecal samples. Most diagnostic laboratories adopt some arbitrary criterion for the numbers of oocysts the presence of which in a gramme of faeces indicates infection sufficient to cause disease, but such a diagnosis should never be made without an adequate case history. Perfectly healthy animals may frequently carry coccidia in numbers sufficient to give a count of several thousand oocysts per gramme of faeces. Conversely, very heavy infection, associated with acute disease may be made manifest before any oocysts can be demonstrated in the faeces. The presence of merozoites or schizonts may then be demonstrated by staining a scraping taken from the intestinal mucosa, with Giemsa.

Prognosis

Up till 1940, no really satisfactory treatment was known for any type of coccidiosis. Since that time, knowledge of satisfactory therapeutic agents has improved very considerably. Treatment is still, however, primarily on a flock or herd basis. Most animals in a group can be treated successfully if therapy commences before the disease becomes established, the first affected animals indicating the time to commence treatment. It is, however, doubtful whether established disease is much affected by the usually recommended drugs, particularly when there is extensive damage to the intestinal mucosa.

The prognosis for the successful control of epidemic group infection is usually excellent. For individual affected animals it is by no means as good.

Treatment

As has been indicated, treatment is on a group basis, all animals likely to have been in contact with the disease being treated directly symptoms begin to appear. The two sulphonamides, Sulphamezathine (sulphadimidine) and sulphaquinoxaline; a mixture of sulphaquinoxaline with pyrimethamine, and a soluble preparation of nitrofurazone and furazolidone (Bifuran) have all been shown to have a marked coccidiostatic

effect. All should be used at a concentration which ensures an inhibitory effect on the second generation schizonts while interfering as little as possible with the earlier development of the parasite. As has been suggested by Kendall and McCullough (1952) for *E. tenella* the effect of the drug is only inhibitory, the resistance of the animal developing during the period when the growth of the parasite is halted by the action of the drug. With this in mind the drugs should be used at as low a concentration as is effective in preventing damage from the growth of the schizonts and preferably in intermittent treatments which permit development of the earlier stages and hence allow the production of immunity.

Of the recommended drugs, *sulphaquinoxaline* is effective at a much lower concentration than *Sulphamezathine* but is also far more toxic. For chickens *sulphaquinoxaline* can be toxic when used at the lowest concentration likely to control acute disease. Bifuran at the concentrations ordinarily recommended is far less effective than *Sulphamezathine* but it is also less likely to induce toxicity.

The sulphonamides are usually given as the soluble sodium salts.

With all the coccidiostatic drugs recommended concentrations should never be exceeded both because of the danger of inducing toxicity and because there is a likelihood of interfering with the immunological processes and thereby reducing the efficacy of treatment.

Recommended drugs for poultry

(1) *Sodium Sulphamezathine*. 0.2 per cent. of the drinking water for a maximum period of six days.

(2) Mixtures of *sulphaquinoxaline* with *pyrimethamine*.

(3) *Sodium sulphaquinoxaline* in the drinking water at a level of 0.043 per cent. for a maximum period of five days (not recommended for chickens four to ten weeks old).

Prevention

As has been indicated, the coccidia are not usually responsible for serious disease unless conditions of management are such that mass infection is favoured, particularly in immature susceptible

stock. It is clear, therefore, that means should be devised to avoid exposing young stock to a high rate of infection. Infection must be contracted gradually so that animals become immune without exhibiting the clinical symptoms of disease. Overcrowding must be avoided and faecal material removed regularly. Young stock should not ordinarily be placed on ground recently vacated by a similar age group, particularly if there is reason to believe that there has been even a mild incidence of clinical coccidiosis. Under some special circumstances, *e.g.* the deep litter system of poultry management, continuous medication of the food, or medication for periods while the stock is regarded as particularly susceptible to heavy infection has been recommended as a substitute for conventional hygiene under the older systems of management. It seems unlikely, however, that continuous treatment can be regarded as a substitute for good management and it seems far more important to ensure that housing is properly designed. The main effect of fermentation in litter is to promote dryness in the surface layers thereby reducing the numbers of oocysts which successfully sporulate.

It will be appreciated that it is quite impracticable, indeed it is inadvisable, to attempt to avoid all infection with coccidia. In the event that epidemic disease arises, gross contamination can be removed from animal houses by thorough scrubbing with hot detergents or with soda, soap and water. Ammonia in solution has a marked lethal effect on oocysts but needs to be applied at a concentration of at least 10 per cent. after the removal of superficial dirt.

Drugs for the prevention of coccidiosis

A large number of drugs has been introduced for the continuous medication of food.

Amprolium : usually given at a concentration of 0.0125 per cent. of the food ; 0.006 per cent. to 0.008 per cent. used in special circumstances.

Antibiotics : *e.g.* chlortetracycline. Generally used at 50 or 100 g./ton. Under stress conditions 200 g./ton would have some coccidiostatic effect: 800 g./ton is needed for a full therapeutic effect and this is too costly. There is apparently a synergistic effect with sulphonamides.

Bifuran : (0.0055 per cent. nitrofurazone and 0.0008 per cent. furazolidone) to give a total concentration in the food of 0.0063 per cent. Double this concentration can be given under special circumstances, *e.g.* exceptionally heavy challenge.

Nicarbazin : given at 0.0125 per cent. of the food.

Nitrofurazone : given at 0.005 per cent. to 0.01 per cent. of the food.

Sulphaquinoxaline : 0.0125 per cent. of the food.

Whitsyn-10 : (sulphaquinoxaline and pyrimethamine in synergistic combination) : given as recommended by the manufacturers.

COCCIDIA OF FOWLS

General characters

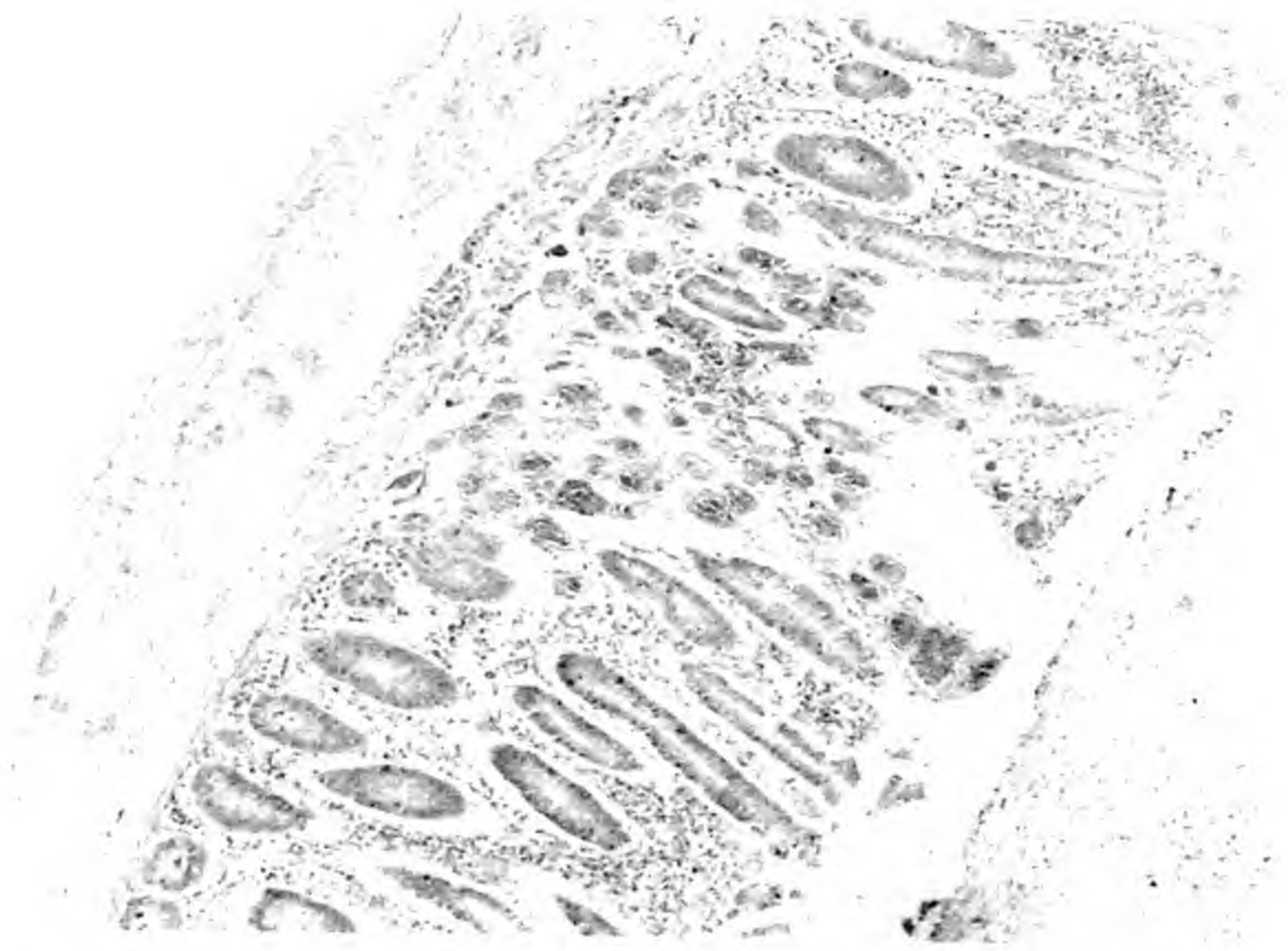
The general characters of the oocysts of species commonly parasitising fowls are given in the Table (I). Of the species listed *E. tenella*, *E. acervulina* and *E. necatrix* are the principal pathogens.

EIMERIA TENELLA (Railliet and Lucet, 1891)

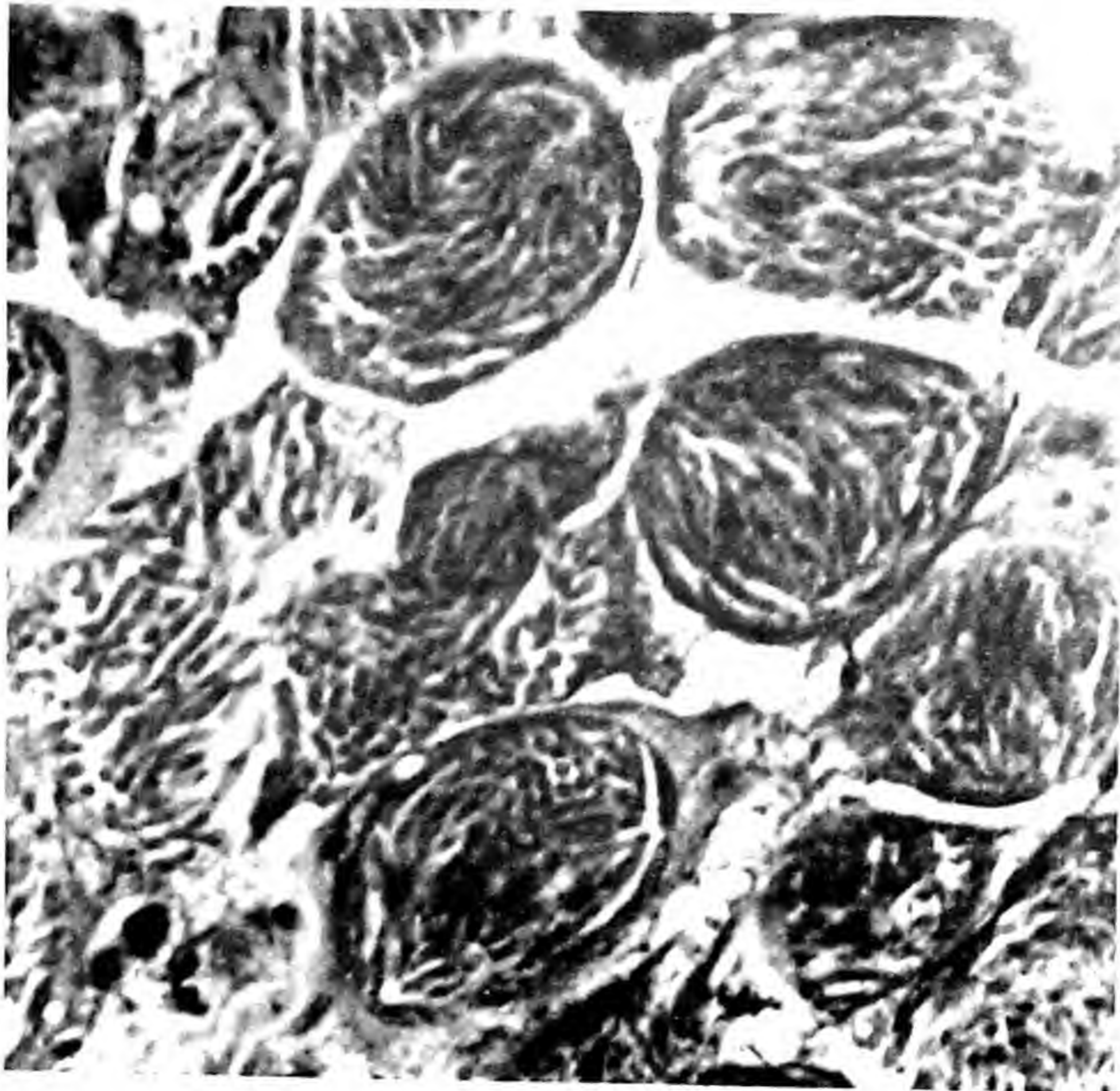
This coccidium is the commonest and the most pathogenic of the coccidia of fowls and it is known as one which is regularly associated with outbreaks of acute disease with a high mortality rate. Young chicks up to the age of about eight weeks are those usually affected with a peak incidence round about five weeks of age. In uncontrolled acute outbreaks the mortality may reach 80 to 90 per cent. The parasite is confined to the cæca and the maturation of the second generation schizonts causes severe hæmorrhage occurring on the fifth day following infection. If the chick survives for 48 hours following the hæmorrhage, it usually recovers, although the persistence of a necrotic core in the cæca may sometimes be associated with a failure to thrive for a period of several months. If the chick survives the bleeding phase oocyst production is apparent on the seventh day following infection, rises to a peak on the tenth to eleventh days and then decreases.

Resistance to further infection starts to develop within a few hours of the appearance of the second generation schizonts and it is very rare for a chick to show clinical symptoms of disease on more than one occasion.

FIG. 21(a)



Eimeria tenella :—showing breakdown of caecal mucosa from maturation of second generation schizonts



Eimeria tenella :—mature second generation schizonts with merozoites (high magnification)

FIG. 21(b)



Eimeria tenella :—chick caeca filled with blood



Eimeria tenella :—advanced breakdown of caecal tissue with bleeding into the lumen : very numerous schizonts

Diagnosis

Diagnosis is made on the presence of blood in the droppings of infected birds. On post-mortem examination the cæca are found to be filled with blood: later with a cheesy-necrotic core.

Treatment

Sodium sulphamezathine given in the drinking water at a concentration of 0·2 per cent. is usually very effective. Treatment

TABLE I. OOCYSTS OF FOWLS

Species	Shape	Size μ	Characteristics
<i>E. acervulina</i>	ovoidal	19 × 14	inconspicuous micropyle
<i>E. brunetti</i>	ovoidal	26 × 20	...
<i>E. hagani</i>	ovoidal	19 × 17	...
<i>E. maxima</i>	ovoidal	30 × 20	large oocyst with yellow wall
<i>E. mitis</i>	subspherical	16 × 15	...
<i>E. necatrix</i>	ovoidal	20 × 17	...
<i>E. præcox</i>	ovoidal	21 × 17	...
<i>E. tenella</i>	ovoidal	23 × 19	...

is on a flock basis and must be started as soon as any disease becomes apparent. Usually blood in the droppings is the first evident sign although some experienced farmers believe they can detect droopiness in the chicks a few hours before bleeding starts. Both sulphaquinoxaline and Bifuran are also effective in checking the disease.

The best type of treatment is the 3-2-3 schedule, whereby the chicks receive two three-day periods of treatment separated by two days without treatment.

EIMERIA ACERVULINA Tyzzer, 1929

This coccidium chiefly inhabits the upper part of the small intestine, the masses of oocysts appearing as white or grey transversely elongated spots on the surface of the intestinal mucosa, particularly the duodenal loop. The oocysts are characterised

by a thinning at the more pointed end with a slight elevation at the margin and they may appear in the fæces on the fourth day following infection. This prepatent period is markedly shorter than that of most of the other coccidia of fowls. *E. acervulina* is found superficially in the epithelial cells and its pathogenicity is nothing like as great as either *E. tenella* or *E. necatrix*. Nevertheless, it appears to be responsible for considerable weight loss and, as the life-cycle may be prolonged over a period of several weeks, *E. acervulina* can be regarded as one of the coccidia chiefly associated with the wasting type of intestinal coccidiosis. Disease appears, as a rule, among birds older than those characteristically affected by *E. tenella*.

Treatment

The coccidiostatic drugs previously recommended for the treatment of chicks affected with *E. tenella* can be used for the treatment of birds affected with *E. acervulina*, but present knowledge suggests that the results of treatment are far less satisfactory. As the prepatent period is so short, interrupted therapy is not likely to be more satisfactory than a period of continuous treatment.

EIMERIA NECATRIX Johnson, 1930

This coccidium is the most pathogenic of the species which comprise the mixed infection usually involved in the less acute wasting type of intestinal coccidiosis. The development of the species is very similar to that of *E. tenella*; the prepatent periods are the same, oocysts appearing first on the seventh day following infection. Oocysts are produced in the cæcal tubes. Pathogenesis is associated with the development of the second generation schizonts which are produced in the small intestine. Their growth results in a severe mucoid enteritis with some hæmorrhage which is, however, usually not so characteristic of the disease as with *E. tenella*. Clinically, the disease is identified by the well-defined round spots which consist of colonies of developing schizonts and which appear on the wall of the small intestine. In cases of severe infection death may occur on the fifth to seventh day following infection but chronic disease is probably more common. The oocysts of *E. necatrix* are often very similar to those of *E. tenella*.

Treatment

Sulphamezathine, sulphaquinoxaline, mixtures of sulphonamides and potentiating drugs and Bifuran all inhibit the development of the parasite, but, as with *E. acervulina* the final effect of treatment on an infected flock remains in doubt. Very often treatment is not commenced until the prepatent period has been completed. By this time the major damage to the intestinal mucosa has occurred.

EIMERIA MAXIMA Tyzzer, 1929

This species, readily detected by the large size of the oocysts, is becoming of increasing importance, particularly in poultry kept under intensive conditions. A moderately severe hæmorrhagic type of coccidiosis is induced, with mucus often coloured pink and flecked with blood, in the droppings. Damage to the intestinal wall is caused by the development of the sexual stages of the parasite, the schizonts being produced superficially in the epithelium, whereas the gametocytes penetrate into the deeper parts of the epithelial cells below the nuclei and project into the subepithelial layers.

OTHER SPECIES OF *EIMERIA* IN FOWLS

The other species listed on page (113) have at various times been reported as being associated with disease. Their precise role in relation to clinical disease requires further investigation. Most outbreaks of disease in the field are associated with several species of *Eimeria* and it is best to give a diagnosis of coccidiosis only if parasites found in large numbers in the intestinal mucosa are associated with a catarrhal enteritis. If *E. necatrix* can be identified, then it is almost certainly responsible for a significant amount of disease. As already indicated, there are well-tried systems of control for *E. tenella*. For the other species, treatment with sulphadimidine or sulphaquinoxaline or with potentiated sulphonamides is likely to give the best results.

COCCIDIA OF TURKEYS

Of the seven species of coccidia described in the turkey (Moore 1954,) two, *E. adenæides* and *E. meleagritidis* are considered to be of significant pathogenicity. Clarkson (1960) has

discussed the species differentiation of *E. adenæides*, *E. meleagrimitis* and *E. meleagridis* which are usually regarded as indistinguishable on the morphology of the oocyst alone.

The oocysts of all three species have a refractile granule and none shows typical residual bodies. There is not sufficient variation in the prepatent periods to allow differentiation to be made :—(*E. adenæides* (114-132 hours), *E. meleagrimitis* (114-118 hours) and *E. meleagridis* (108-112 hours).)

Pathogenicity

E. adenæides and *E. meleagrimitis* are very pathogenic whereas *E. meleagridis* is non-pathogenic.

Distribution and morphology of the development forms

(a) *E. meleagrimitis* is mainly localised in the duodenum and the upper small intestine ; *E. meleagridis* has first stage schizonts in the middle small intestine but later stages in the cæca only. *E. adenæides* is in the lower small intestine, cæca and rectum.

(b) In the case of *E. meleagrimitis* the first stage schizonts are always found in the glands of the duodenum, where the sexual stages are never found. In *E. adenæides* and *E. meleagridis* the first stage schizont is rarely found in the glands, whereas the sexual stages, especially in *E. adenæides*, occur both in the glands and in the tips of the villi.

(c) The first stage schizonts of all the three species develop deep beneath the cell nucleus whereas the other stages are nearly always found above the nucleus.

The gross pathology provides a rapid and reliable means of distinguishing between the three species. It is the sexual stages that produce disease.

E. adenæides induces the appearance of white material composed of oocysts, gametocytes and cellular debris in the lower small intestine and cæca.

E. meleagrimitis induces a necrotic lesion in the duodenum and upper small intestine.

E. meleagridis induces the appearance of yellow caseous

material localised in the cæca only. These lesions are well marked but in fact are not of great pathological significance.

TABLE II OOCYSTS OF TURKEYS

Species	Shape	Size μ	Characteristics
<i>E. adenæides</i> .	ellipsoidal	26×17	up to 3 polar granules
<i>E. dispersa</i> .	ovoidal	26×21	...
<i>E. gallopavonis</i> .	ellipsoidal	27×17	single polar granule
<i>E. innocua</i> .	subspherical	22×21	no polar granules
<i>E. meleagridis</i> .	ellipsoidal	24×18	1 or 2 polar granules
<i>E. meleagrimitis</i> .	subspherical	20×17	single polar granule
<i>E. subrotunda</i> .	subspherical	22×20	no polar granules
<i>C. meleagridis</i> .	?	4.5×4	indistinct, foam-like cytoplasm

Immunity

There is an age resistance to the clinical effects of infection with *E. meleagrimitis*; poults over eight weeks of age being able to withstand exposure to comparatively heavy infection. All species appear to elicit at least some degree of resistance to reinfection.

Treatment

Sulphaquinoxaline at a concentration of 0.0125 per cent. in the food, fed to poults up to eight weeks of age is highly effective in preventing disease arising from the infection with either of the pathogenic species. Toxicity arising from the use of the drug for turkeys has not so far been reported.

Nitrofurazone at a concentration of about 0.01 per cent. of the food for a period of ten days is reported to have been used successfully in controlling mild outbreaks of disease.

COCCIDIA OF GESE

Four species of *Eimeria* and one species of *Tyzzeria* have been described from the domestic goose.

TYZZERIA ANSERIS Nieschulz, 1947

Farr and Wehr (1952) have given an account of severe disease in young goslings which were, however, infected also with

Eimeria truncata. Later Hanson, *et al.* (1957) found that *T. anseris* was the most widely distributed coccidium of wild geese in North America. They considered it to be primarily a parasite of the wild goose. It occurs only rarely in domesticated birds.

EIMERIA TRUNCATA Railliet and Lucet, 1890

This species of *Eimeria* is of particular interest as it occurs in the kidney of the goose. The parasites are found in the epithelium of the kidney tubules, considerable disruption of the tissue resulting. The oocysts are truncate at the anterior end and measure about $25\ \mu \times 15\ \mu$. There is a residual body in the sporulated oocyst.

Mortality among young geese may be quite extensive, post-mortem examination showing considerable enlargement of the kidneys which are light in colour with small nodules throughout the kidney substance.

Treatment

Sodium sulphamezathine or sodium sulphaquinoxaline as recommended for chickens.

OTHER SPECIES IN THE GOOSE

Other species, as listed in the Table III, have been described from the goose. Little is known about their life histories and their pathogenicity is in doubt.

TABLE III. OOCYSTS OF GEESE

Species	Shape	Size μ	Characteristics
<i>E. anseris</i>	pyriform	22×17	colourless wall, prominent micropyle
<i>E. brantæ</i>	ovoidal	23.4×17.7	colourless; prominent micropyle
<i>E. magnalabia</i>	ovoidal	$21.7-24 \times 15.1-17.3$	yellow-brown wall, $1.8\ \mu$ thick, prominent micropyle with thick lips
<i>E. nocens</i>	ovoidal, flattened at micropylar end	31×22	yellow-brown wall, prominent micropyle
<i>E. parvula</i>	ellipsoidal	$10-15 \times 10-14$	colourless
<i>E. truncata</i>	ellipsoidal, truncated at micropylar end	21×17	colourless wall, prominent micropyle
<i>T. anseris</i>	ellipsoidal	$12-16 \times 10-12.5$	colourless

TABLE IV. OOCYSTS OF PHEASANTS

Species	Oocysts		Special characters
	Average size	Shape	
<i>Eimeria phasiani</i> . Tyzzer, 1929	23·0 × 16·0 μ	Long ellipsoidal	Has polar granule
<i>E. dispersa</i> . Tyzzer, 1929	20·0 × 16·0 μ	Ovoid	No polar granule

Some species in pheasants invade the epithelium of the small intestine and may be very pathogenic. There are a few references in the literature *e.g.* Ormsbee (1939).

COCCIDIOSIS IN DUCKS

Occasional outbreaks of coccidiosis, occurring in flocks of young ducks, appeared to be controlled by the use of sulphamezathine. There is little information about the species involved. Davies (1957) has reported the occurrence of *Tyzzeria perniciosa*.

COCCIDIA OF THE RABBIT

E. stiedæ*, *E. perforans*, *E. media*, *E. magna*, *E. irresidua

General characters. The parasites are nearly always found in mixed infections. The level of infestation in apparently healthy rabbits is very high. All the oocysts except those of *E. perforans* have a visible micropyle. An oocystic residual body is absent in *E. stiedæ* and *E. irresidua* but present in the other three. In *E. magna* the oocyst wall at the micropyle end is thickened to form a kind of collar and the oocysts are brownish in colour.

E. STIEDÆ (Lindemann, 1865)

This pathogenic coccidium parasitises the liver which becomes enormously enlarged with characteristic white or yellowish-white areas round the dilated bile ducts. Here oocysts can be found in enormous numbers. They begin to reach the exterior via the faeces on about the fifteenth day following infection. The faecal

oocyst count rises rapidly to a peak by about the twenty-second day and then decreases. The animal then appears to be resistant to further clinical disease and the life-cycle of the parasite probably

TABLE V. OOCYSTS OF RABBITS

Species	Shape	Size μ	Characteristics
<i>E. irresidua</i>	ovoidal	38×26	yellow wall, prominent micropyle; no oocystic residual body
<i>E. magna</i>	ovoidal	36×24	yellow brown wall, prominent micropyle with lip-like elevations; large oocystic residual body
<i>E. media</i>	ellipsoidal	31×18	pink colour, prominent micropyle
<i>E. perforans</i>	ellipsoidal	23×14	colourless, no micropyle
<i>E. stiedæ</i>	ellipsoidal	37×20	smooth, light-yellow wall; wide, thin micropyle

terminates, although oocysts may be mechanically retained and excreted for a considerable period of time. Death may occur as early as the twelfth day following infection or it may be very considerably later.

THE INTESTINAL COCCIDIA OF RABBITS

The parasites are rarely found in pure infection and their separation and experimental investigation in pure line is technically difficult. The most noticeable feature on post-mortem examination is the marked dilatation of the intestine. The wall of the small intestine is œdematous and covered with an excess of reddish mucus. When the mucus is washed away, white spots or streaks represent areas of oocyst formation. The capillaries are congested and hæmorrhage may occur. Of the intestinal forms, *E. perforans* and *E. magna* are usually regarded as the most pathogenic. Rutherford (1943) made a study of the intestinal coccidia of rabbits and found that animals given heavy doses of infective oocysts show no symptoms until about 24 hours before oocysts appear in the fæces. Heavy infections cause violent diarrhœa which often results in death. According to Rutherford, *E. magna* and *E. irresidua* are the most pathogenic species, causing necrosis of the

parasitised cells. All the intestinal species except *E. magna* complete their development throughout the length of the small intestine. *E. magna* does not inhabit the first 12 inches of the gut. *E. irresidua* seems to occur principally in the first 18 to 24 inches of the gut.

It is now considered that so-called *nasal coccidiosis* is due to the spurious presence of oocysts on the nasal mucous membrane, possibly as a result of the coprophagy practised by all normal healthy rabbits.

Control

Sulphamezathine given in the drinking water to rabbits which are on an all-pellet diet, at a concentration of about 0.2 per cent., is effective in controlling coccidiosis. If the rabbits are being fed succulent green food powdered Sulphamezathine or the sodium salt may be incorporated in a small wet mash at the rate of 0.75 gm. per day for five days for an average-sized rabbit. Under experimental conditions rabbits have been given such amounts of drug for several months without any evident deleterious effect.

Prevention

There are probably only two really satisfactory ways of rearing young rabbits without loss from coccidiosis. They may be maintained on stout wire or hard cloth mesh which is in part self-cleaning and can in addition easily be sterilised, or they can be reared on clean pasture in wire-bottomed houses which are moved at least every other day. Wild rabbits must under such conditions be excluded.

COCCIDIA OF SHEEP AND GOATS

There is comparatively little information regarding the coccidial infections of sheep and goats but a number of species has been described by Christensen (1938) and Honess (1942).

Becklund (1957), Shumard (1957 and 1959), Salisbury and Whitten (1953), Salisbury, Muir and Stirling (1953) and Whitten (1953) have described outbreaks of disease.

Most work has involved the observation of natural infections under field conditions where controlled observation was difficult. As a rule, flocks of adult sheep appear to carry a light infection with coccidia and disease occurs among lambs which are run with them or which follow them closely onto the same ground.

TABLE VI. OOCYSTS OF SHEEP AND GOATS

Species	Shape	Size μ	Characteristics
<i>E. ah-sa-ta</i>	ellipsoidal	33×24	faint pink colour
<i>E. arloingi</i>	ellipsoidal	27×18	prominent polar cap
<i>E. crandallis</i>	ellipsoidal	22×19	...
<i>E. faurei</i>	ovoidal	29×21	distinct micropyle
<i>E. granulosa</i>	urn-shaped	29×21	brownish yellow wall, large flat polar cap
<i>E. intricata</i>	ellipsoidal	47×32	thick brown wall transversely striated, prominent polar cap and micropyle
<i>E. ninæ-kohl-yakimovæ</i>	ellipsoidal	23×18	...
<i>E. pallida</i>	ellipsoidal	14×10	...
<i>E. parva</i>	subspherical	16×14	...
<i>E. punctata</i>	subspherical	21×18	prominent polar cap and micropyle. External surface marked with cone-shaped pits

Symptoms and pathology

The species often associated with disease is *E. arloingi*. Acute cases exhibiting few symptoms beyond abdominal pain may occur with death following in a day or two. More often profuse diarrhœa occurs with resulting emaciation. Christensen and Foster (1943), working with experimentally infected lambs, found that symptoms commenced on the twelfth day with a peak of severity on the fifteenth day and recovery by the twenty-second day. In two animals quantities of blood appeared in the fæces. In some animals as many as 100,000 oocysts per ml. of fæces were recorded.

The mucous membrane of the small intestine is thickened and œdematous and covered with a muco-fibrinous deposit. The capillary vessels are congested and hæmorrhages may occur. In the more chronic cases the epithelium of the small intestine proliferates under the influence of the multiplication of the parasite and papilliform elevations about a centimetre long may be formed. They consist of a core of glandular tissue covered with stratified epithelium.

Treatment

Sulphamezathine given as a dose of 0.1 gm./kilo for five days has proved successful for the control of clinical disease under

field conditions, although Whitten (1953) has had disappointing results.

COCCIDIOSIS IN SWINE

General characters. The oocysts have no visible micropyle, there is no oocystic residual body, but a sporocystic residual body

TABLE VII. OOCYSTS OF SWINE

Species	Shape	Size μ	Characteristics
<i>E. debliccki</i> .	ovoidal	21 × 16	colourless, smooth wall
<i>E. perminuta</i> .	subspherical	11·2-16 × 9·6-12·8	yellow rough wall
<i>E. polita</i> .	ellipsoidal	22-31 × 17-22	light brown, wall less rough than <i>E. scabra</i>
<i>E. scabra</i> .	ellipsoidal	22·4-35·6 × 16-25·6	rough brown wall
<i>E. scrofae</i> .	cylindrical	24 × 15	distinct micropyle
<i>E. spinosa</i> .	ovoidal	16-22·4 × 12·8-16	brown wall, surface studded with spines
<i>I. suis</i> .	subspherical	23 × 19	yellow, smooth surface

occurs. *E. debliccki* is said to be polymorphic in respect of the size of its oocysts, which occur in large, small and intermediate forms. It is considered that these forms represent one species, as by feeding single oocysts both large and small forms reappear on infection. (The author, however, noted, in inducing sporulation, that the small forms sporulate within a week whilst in the large forms sporulation may be delayed for three weeks.)

Incidence and pathogenicity

E. debliccki and *Isospora suis* are the commonest forms encountered. Disease is not commonly recorded in Britain, but in the United States of America *E. debliccki* is said to cause disease in young pigs, the parasites attacking the mucous membrane of the small intestine, causing catarrhal and hæmorrhagic inflammation with distension of blood vessels (Biester and Murray, 1929).

COCCIDIA OF CATTLE

Serious illness in cattle as the result of infection with coccidia is not reported very commonly in Europe but it is possible that a number of the unidentified cases of enteritis among calves may in fact be due to this cause.

In the United States of America coccidiosis has been listed as the third most important parasitic disease in cattle (Swales *et al.*, 1948). Most reports indicate that the disease occurs in

TABLE VIII. OOCYSTS OF CATTLE

Species	Shape	Size μ	Characteristics
<i>E. alabamensis</i>	pyriform	19×13	sporocysts have parachute-shaped cap
<i>E. auburnensis</i>	ovoidal	38×23	yellowish-brown wall, prominent micropyle
<i>E. azerbaijdzhana</i>	cylindrical or bean-shaped	45×22	...
<i>E. böhmi</i>	ellipsoidal	$34-49 \times 24-33$	prominent polar cap
<i>E. bombayensis</i>	ellipsoidal	37×22	well defined micropyle 2-4 μ in diameter
<i>E. bovis</i>	ovoidal	28×20	micropyle is a lightened area in cyst wall
<i>E. brasiliensis</i>	ellipsoidal	38×27	prominent polar cap and micropyle
<i>E. bukidnonensis</i>	pyriform	44×32 (Lee, 1954)	yellow-brown wall, 2 μ thick with radial striations
<i>E. canadensis</i>	ellipsoidal	33×23	inconspicuous micropyle
<i>E. cylindrica</i>	cylindrical	23×14	thin, transparent cyst wall
<i>E. ellipsoidal</i>	ellipsoidal	17×13	thin, transparent cyst wall
<i>E. ildefonsoi</i>	ovoidal	42×28	smooth, thick brown wall
<i>E. khurodensis</i>	ellipsoidal	$40-44 \times 28-30$	thick brown wall with mammillations, micropyle up to 9 μ wide
<i>E. mundaragi</i>	ellipsoidal	37×27	thin yellow wall with distinct micropyle
<i>E. pellita</i>	ovoidal	40×28	thick brown wall with fine projections
<i>E. subspherica</i>	subspherical	11×10	thin, transparent wall
<i>E. thianethi</i>	ellipsoidal	43×29	thick yellow wall with transverse striations
<i>E. wyomingensis</i>	ovoidal	40×28	yellow-brown wall with conspicuous micropyle
<i>E. zurnii</i>	subspherical	18×16	smooth, colourless transparent wall

epidemic proportions among calves during the autumn and winter. In the tropics the disease is commonly reported, particularly as the result of the mustering associated with mass inoculations for rinderpest. Lee and Armour (1959) have surveyed the species of coccidia occurring in cattle in Nigeria. Throughout the world acute disease is usually associated with either *E. bovis* or *E. zurnii*. Heavy infection with *E. auburnensis* or *E. alabamensis* may cause some pathogenic effects, at least under experimental conditions.

Symptoms and lesions

These include debility, abdominal pain, loss of appetite, and diarrhœa with excess mucus and blood clots. The mucous membrane of the affected bowel is thickened, œdematous and covered with muco-fibrinous deposit. The epithelial layer may be desquamated and hæmorrhages may occur from the congested capillaries. Secondary infection may result in the production of necrotic ulcers.

The symptoms shown by calves infected experimentally with *E. bovis* have been described by Hammond and others (1944). No abnormality was noted for 17 days. On the following day diarrhœa was observed and the fæces were streaked with blood. Symptoms progressed until the twenty-fifth day. Post-mortem examination showed that marked lesions were confined to the lower ileum, cæcum and colon, which were swollen and thickened and contained semi-fluid bloody material. It appeared that the sexual stages of development of the parasite were responsible for the tissue damage.

E. zurnii is probably the most pathogenic of all the bovine species and is associated with very many of the outbreaks of clinical disease in the field. Davis and Bowman (1957) infected calves aged two days to eight weeks with a large number of oocysts and slaughtered them at intervals in order to determine the course of the infection. Trophozoites were seen on the second and third days after infection. They penetrated the tissues of the small intestine, some penetrating down to the *muscularis mucosæ*. Schizonts were seen on the sixth day and until the nineteenth day.

It is possible that more than one asexual generation occurs. The major lesions occur in the large intestine, sexual and asexual

stages of the parasite occurring together, invading the glands of Lieberkuhn and inducing sloughing of the epithelium. Hæmorrhage takes place from the exposed capillaries, masses of fibrin, leucocytes and red blood corpuscles may adhere to the denuded area and these masses may contain large numbers of oocysts. The sloughing of the epithelium and the necrosis are not seen in typical infections with *E. bovis* where endothelial cells lining the lacteals of the villi also are invaded, causing eventual distension and possible rupturing of the tips of the villi by the schizonts.

Diagnosis

Experience in Britain suggests that the presence of oocysts in appreciable numbers (more than 50,000 per ml. of liquid fæces) together with a history of diarrhœa among young cattle should be regarded as indicative of clinical coccidiosis. Oocysts in large numbers are not found as commonly in the fæces of cattle as in those of some other healthy animals.

Treatment

Treatment with sulphamezathine, giving an initial dose of 0.2 gm. per kilo body weight followed by half this dose for a further four days appears to give satisfactory control under field conditions.

COCCIDIOSIS IN CARNIVORES

In carnivora the commonest species of coccidia belong to the genus *Isospora*. These parasites are believed not to show the marked host-specificity of the *Eimeria*. Whilst the species in each animal have been described under different names, there appears to be no evidence, *e.g.* that the species of *Isospora* occurring in cats, foxes and mink are not the same as those occurring in dogs.

Pathogenicity and Treatment

The asexual forms of all the species attack the mucous membrane of the small intestine, but appear to have little pathogenicity. *I. bigemina* has been described as causing diarrhœa in dogs, and in mink heavy mortality has been ascribed to coccidiosis. Parkin (1943) claimed good results in the treatment of canine

coccidiosis with an enema of a 1 per cent. solution of sodium sulphanilyl—sulphanilate using 10 c.c. per 1 kg. body weight repeated after 24 hours. In cats, Brumpt (1943) found mepacrine in doses of 0.01 gm. per kg. effective.

TABLE IX. OOCYSTS OF CARNIVORES

	Oocyst		Notes
	Average size	Shape	
<i>I. bigemina</i> . . . (Polymorphic) Stiles, 1891	(12.0 × 8.0 μ 19.0 × 15.0 μ	Cylindrical	Stated that sporulation may occur in the intestines
<i>I. rivolta</i> . . . Grassi, 1879	23.0 × 17.0 μ	Egg-shaped	
<i>I. felis</i> . . . Wenyon, 1923	45.0 × 33.0 μ	Egg-shaped	
<i>E. canis</i> (dog) . . Wenyon, 1923	28.0 × 16.0 μ	Ellipsoidal	Micropyle just visible
<i>E. vulpis</i> (fox) . . Galli-Valerio, 1929	18.0 × 12.0 μ	Ovoid	
<i>E. felina</i> (cat) . . Nieschulz, 1924	23.0 × 15.0 μ	Ellipsoidal	
<i>E. cati</i> (cat) . . . Yakimoff, 1933	20.0 × 17.0 μ	Ovoid	Yellowish-brown inner layer of oocyst wall. No micropyle
<i>E. mustelæ</i> (mink) . Kingscote, 1935	20.5 × 14.5 μ	Egg-shaped	

There has in fact been very little critical work on the coccidiosis of carnivores, and little is known about the chemotherapy. One would expect the sulphonamides to be effective as in other animals. Duberman (1960) in an uncontrolled experiment noted that both nitrofurazone and a mixture of sulphonamides were effective in the treatment of dogs infected with *Isospora felis*.

COCCIDIOSIS IN CAMELS

There are few reports in the literature. Abdussalam and Rauf (1957) found infection in 10 of 24 camels in Lahore. There was no evidence of disease.

TABLE X. OOCYSTS OF CAMELS

Species	Size	Shape	General characters
<i>Eimeria cameli</i> . Nöller, 1932	30.0 × 24.0 μ	Oval	Micropyle and polar cap

COCCIDIOSIS OF OTHER ANIMALS

Species of *Eimeria* occur in a wide range of mammalian and avian hosts. A check list of species of *Eimeria* recognised up to 1943 is given in the *Proc. Helminth Soc. Wash.*, x, 35.

Later lists have been published by Becker (1956) and Pellérdy (1956 and 1957).

CHAPTER X
SPOROZOA
THE FAMILIES HÆMOPROTEIDÆ
AND PLASMODIIDÆ

LEUCOCYTOZON-HÆMOPROTEUS-PLASMODIUM

THE Family *Plasmodiidæ* contains the genus *Plasmodium* which is of outstanding medical importance as it includes the blood parasites which cause malaria. Schizogony occurs in the peripheral blood of vertebrates and the parasites are characterised by the occurrence of pigment granules.

The Family *Hæmoproteidæ* includes *Hæmoproteus* in which pigmentation is present in the gametocytes. Gametocytes appear in the peripheral blood of the host ; schizogony occurs elsewhere. With the related *Leucocytozoon* the gametocytes do not contain pigment.

Fallis and Bennett (1961) have discussed the development of both *Leucocytozoon* and *Hæmoproteus* and on the basis of their observations suggest that the genera *Plasmodium*, *Leucocytozoon* and *Hæmoproteus* should be placed in separate families of the order *Hæmosporidiida*.

Genus *LEUCOCYTOZON* Danilewsky, 1890

Schizogony occurs in the endothelial cells as well as in the visceral cells of vertebrates. Sexual reproduction occurs in blood-sucking insects, notably species of the genus *Simulium*. O'Roke (1934) observed the development of *Leucocytozoon simondi* in *Simulium venustum*. Gametocytes taken with blood from an infected duck rapidly developed into macrogametes and microgametes (4-8 of which developed from a single microgametocyte). Zygotes developed in the stomach content of the fly and gave rise, through motile forms (ookinetes), to oocysts which were found on the outer wall of the stomach. Sporozoites injected into the duck developed into schizogonic stages in the endothelial cells of the capillaries of lungs, liver and spleen. On about the seventh

day after infection gametocytes appeared in the blood. These occur in different stages of development in degenerate spindle-shaped host cells (possibly leucocytes). The parasites stain blue with Giemsa, with a dark-red nucleus.

The life-history has been further investigated by Fallis, *et al.* (1951).

LEUCOCYTOZOON SMITHI Laveran and Lucet, 1905

This parasite has been recorded from the continent of Europe and also in the U.S.A. and has been held responsible for very

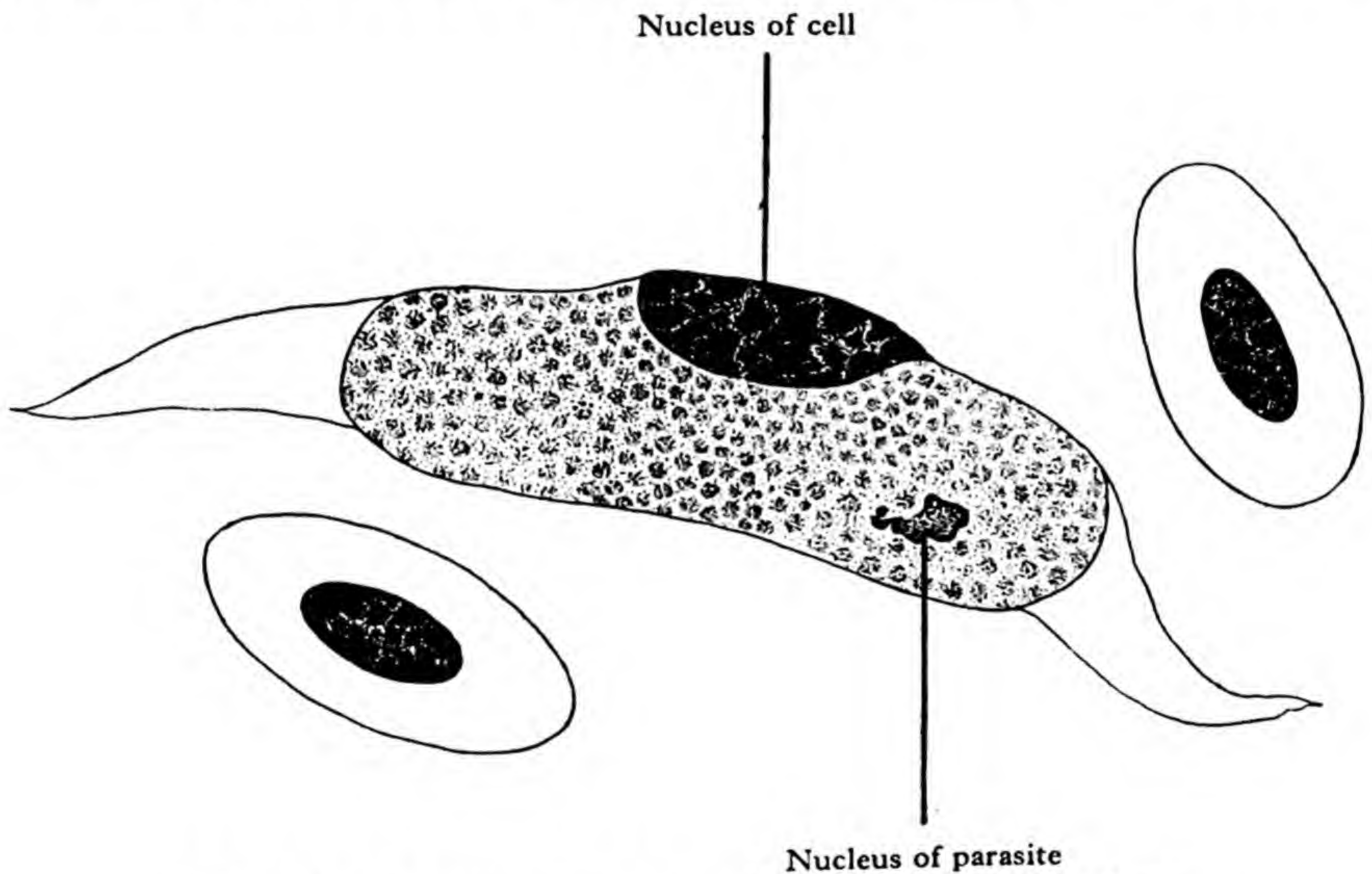


FIG. 22.—*Leucocytozoon neavei* in blood of guinea-fowl.

severe mortality in young turkeys. In one case 350 deaths out of 385 birds were recorded. The symptoms are described as loss of appetite, emaciation, debility and great leg weakness. In moving, the tail is used to assist the legs as a support. Drowsiness is a common symptom, and when the birds are wakened they may go into convulsions. Birds in semi-coma give off a foamy discharge from the beak and nostrils.

Injection, under experimental conditions, of heavily parasitised blood into susceptible poults did not induce disease (Skidmore, 1935).

Lesions

Flesh yellowish and anæmic. Slight œdema of the pro-ventriculus and congestion of the duodenum. Cæca normal. Liver slightly enlarged. Spleen enlarged and congested. Lungs and kidneys slightly congested.

Diagnosis

This is by the symptoms, confirmed by examination of blood smears, and identification of the large ($50\ \mu$ long) spindle-shaped parasitised cells.

TABLE XI. Genus *Leucocytozoon*

Species	Host	Size of parasitised host cell	Size of mature gametocyte
<i>Leucocytozoon smithi</i> (Laveran and Lucet, 1905)	Turkey	$50.0 \times 8.0\ \mu$	$25.0 \times 6.0\ \mu$
<i>L. neavei</i> (Balfour, 1906)	Guinea-fowl	$30.0 \times 10.0\ \mu$	$20.0 \times 10.0\ \mu$
<i>L. caulleryi</i> Mathis and Léger, 1909	Fowl	$20.0 \times 20.0\ \mu$	$15.5 \times 15.0\ \mu$
<i>L. sabrazezi</i> Mathis and Léger, 1910	Fowl	$67.0 \times 6.0\ \mu$	$24.0 \times 4.0\ \mu$
<i>L. simondi</i> Mathis and Léger, 1910 (Syn. <i>L. anatis</i>) Wickware, 1915	Duck	$50.0 \times 10.0\ \mu$	$25.0 \times 5.0\ \mu$
<i>L. anseris</i> Knuth and Magdeburg, 1922	Goose	$15.0 \times 6.0\ \mu$	$6.0 \times 5.0\ \mu$
<i>L. andrewsi</i> Atchley, 1951	Fowl	$15.0 \times 17.0\ \mu$	$11.0 \times 13.0\ \mu$

Treatment

Bierer (1950) has recommended sulphaquinoxaline "at the same concentrations as for other diseases" for the control of the clinical disease in turkeys.

L. SIMONDI Mathis and Léger, 1910

The parasite may cause severe mortality in young ducks (Fallis *et al.*, 1951)

Chernin (1952) (*b*) made observations on natural infection in White Pekin ducklings in Michigan. The termination of the prepatent period was marked by the sudden appearance of

immature parasites in different stages of growth in the peripheral circulation. Morphologically mature gametocytes first appeared on the fifth day after infection. There was a peak density of gametocytes of all ages between the third and ninth day of patency. Peak parasite counts could reach as many as 1000 gametocytes per 10,000 red cells (using these as an indicator). The primary parasitæmia usually terminated within 30 days of the first appearance of parasites.

Epidemiology

Chernin (1952) (a) has investigated the epidemiology of the disease in Northern Michigan. Ducks exposed to infection in the field in late June or early July did not become infected at all rapidly, nor did all birds become infected whereas infections were very rapidly acquired during July and August. By mid-August the epizootic had declined very greatly. Birds introduced to farms where the disease was endemic suffered three to five times the mortality rate of the indigenous farm birds, this suggesting that light infection acquired by young birds early in the season conferred some degree of resistance. However, older birds (18 weeks of age) seemed to survive primary infection rather better than younger birds.

Chernin (1952) (a) further investigated an exceedingly interesting relapse phenomenon in domestic ducks. Ducks affected with *L. simondi* tend to relapse in the spring and many parasites appear in the peripheral circulation. This relapse seems to be associated with the onset of sexual activity in the duck and if by the use of artificial light the time of egg-laying is altered at the same time parasitic relapse occurs.

Genus *HÆMOPROTEUS* Kruse, 1890

HÆMOPROTEUS COLUMBÆ (Celli and Sanfelici, 1891)

This organism occurs in pigeons in South Europe, Africa, India and South America. Similar parasites occur in guinea-fowl and other birds. Schizogony occurs in the endothelial cells of various organs, but is best seen in lung smears. The invaded cells become considerably enlarged and in them are seen a number of multinucleate bodies, each of which appears to be surrounded

with a fine membrane. Within this capsule, the body divides into an enormous number of merozoites. The endothelial cells break down and the cysts containing the merozoites are set free in the capillaries, where the cyst capsule ruptures, setting free minute uninucleate merozoites. The gametocytes are first detectable in the red corpuscles as minute rings with a chromatin granule. As many as a dozen young forms may occur in one cell, but when fully grown it is rare to find more than one parasite present in a corpuscle.

The organisms grow round the cell nucleus, which may be pushed out of position, but the cell is not distorted. When fully formed, the sausage-shaped gametocyte consists of hyaline cytoplasm, staining pale blue with Romanowsky stains, and a large central nucleus containing fine chromatin granules. The female gametocyte has a denser cytoplasm which stains a deeper blue and a more compact nucleus, in which a distinct karyosome can be detected. Both forms of gametocytes contain pigment granules, but those of the male are collected into spherical masses, whilst those of the female are scattered through the cytoplasm.

The gametocytes are taken up with the blood by the invertebrate host, a fly, *Lynchia maura*, in the intestine of which the microgametocytes give rise to microgametes by a process of flagellation. The nucleus breaks up into a large number of chromatin particles, each of which passes into a cytoplasmic process which lashes about like a flagellum. Eventually these processes break loose as uninucleate organisms. The macrogametocyte gives rise to a single macrogamete. After syngamy the zygote develops into a motile ookinete which penetrates the mid-gut of the fly and encysts in the body cavity as a pigmented oocyst. The oocysts increase in size, the nucleus divides and sporozoites are produced. These are liberated and proceed to the salivary glands. As the vegetative forms of the parasite do not occur in the blood, and as the gametocytes do not undergo development till ingested by the invertebrate host, this infection is not transmissible by blood inoculation.

Recent work has suggested that most of the named vectors of *H. columbæ* are synonyms for *Pseudolynchia canariensis* but Baker (1957) has shown that in England the "louse-fly" *Ornithomyia avicularia* may act as vector. Fallis and Wood (1957)

found that *H. nettionis* was transmitted to ducks by nocturnal biting midges (*Culicoides spp.*) The parasite was seen in the peripheral blood of ducks 14-21 days after infection.

Pathogenicity

Two forms of infection due to *Hæmoproteus* are described ; an acute form occurring in young birds and a chronic form in adults. Heavy mortality has been recorded in pigeon nestlings, and the disease may be of importance to breeders of carrier pigeons, but it is doubtful if these serious outbreaks were not complicated by other infections. No satisfactory treatment has been discovered, and control measures should be directed to the destruction of the insect vector. For further reading see Sergeant (1907), Fallis and Wood (1957), Baker (1957) and Fallis and Bennett (1961).

Immunity

Infection may persist for periods of up to three years, and recovered birds are apparently not immune to reinfection.

Genus *PLASMODIUM* Marchiafava and Celli, 1885

This genus is of particular importance because it includes the parasites which cause malaria in man. The disease remains the most important of all those caused by protozoa. Malaria parasites are characterised by their pigment granules and by the schizogony which occurs in the erythrocytes and also in the endothelial cells of man, other mammals, birds and reptiles. Sexual reproduction occurs in blood-sucking insects of numerous species. In man species of *Anopheles* usually act as vectors. A few species, particularly *Plasmodium gallinaceum* of the fowl, are of interest in veterinary medicine. Some other species are of importance because of their use in laboratory animals for the screening of antimalarial drugs and for fundamental work on the problems of malaria in man.

Morphology and life-history

In all species, natural infection commences with the inoculation of the minute spindle-shaped sporozoites by the vector

mosquito. In typical instances these develop in cells of the reticulo-endothelial system into cryptozoites, by assuming a spheroid shape and increasing in size by repeated nuclear division. When this schizogony has been completed the merozoites enter new macrophages and endothelial cells and become metacryptozoites which again undergo schizogony. After three or four generations the merozoites enter erythrocytes and appear in the general circulation. The type of schizogony which occurs in the endothelial cells of liver, heart, spleen and other organs is known as *exoerythrocytic schizogony* (James and Tate (1938)). For a long time only erythrocytic schizogony was known to occur. It could be shown, however, that quinine given in very large doses to canaries on the day of infection failed to prevent the establishment of *Plasmodium relictum* and that when suspensions of the sporozoites of *P. cathemerium* were inoculated into canaries the blood did not become infective for three days, although emulsions of the spleen and other organs contained infectious parasites. The nature of the development during this prepatent period was finally elucidated with bird malaria and as recently as 1948 in mammals. There is a general review of exoerythrocytic development by Garnham (1948). When the merozoites have entered the erythrocytes, schizogony continues and from this point the number of parasitised cells increases progressively. The youngest stages in the blood are small rounded forms containing a large vacuole which displaces the cytoplasm to the periphery of the cell while the nucleus is situated at one of the poles. This gives the young parasite a ring-shaped form. As the parasites grow, they tend to become more irregular in shape. These growing stages of the parasite are the trophozoites. In the course of development yellow-brown to black pigment spots appear in the parasites. These *hæmozoin granules* are apparently katabolic products formed during growth. They vary in form, size and number and have some taxonomic significance. When schizogony again occurs, a variable number of merozoites is formed, the red blood corpuscle bursts and the merozoites and the pigment are liberated into the blood stream. The number of merozoites is fairly constant for each species of malaria and has been used as a means of differentiating the species. In nearly all types of malaria, there is a tendency for the development of all the schizonts

in the blood stream to occur simultaneously so that there is a mass bursting of the erythrocytes. This fact accounts for the periodicity of the paroxysm in the clinical disease, the febrile periods corresponding with the liberation of the merozoites from the schizonts. This is believed to be accompanied by the liberation of poisonous substances into the blood of the host. Merozoites entering the red blood corpuscles repeat the process of schizogony but finally develop into gametocytes. The conditions under which the gametocytes appear are not clearly understood, but it may be in response to the state of the immune reaction of the host. The gametocytes do not undergo any further development until taken up by the invertebrate host, further development being correlated with a lower temperature and also with a change of pH. If living mature microgametocytes of human *Plasmodium* are examined microscopically under a sealed cover-glass at room temperature (18° C. to 22° C.) development takes place in a short while and motile microgametes are produced by a process which has been called *exflagellation*. Similar changes take place in the stomach of a suitable mosquito. When ingested by the invertebrate, the red corpuscle ruptures in the gut and the nucleus of the microgametocyte breaks up into a large number of chromatin particles and the cytoplasm into long processes, into each of which a chromatin particle passes. The residual body of the gametocyte has attached to it four to eight flagella-like processes, the microgametes, which lash vigorously. At this stage the organism is known as a "flagellating body". Each microgamete measures about 20 to 25 μ in length. It soon becomes detached from the residual body and swims freely in the lumen of the stomach of the mosquito. In the meantime, the female gametocyte has undergone a maturation process in the course of which the nucleus is believed to pass through meiotic divisions resulting in reduction of the chromatin. It then forms the mature female gamete (macrogamete). One microgamete enters the macrogamete and syngamy takes place with nuclear fusion. In the insect host, the zygote is at first rounded and motionless but gradually changes to an elongate motile form, the ookinete, which glides about in the intestinal contents. The ookinete penetrates the intestinal wall and comes to rest between the epithelium and the elastic membrane lining the body cavity. Here it contracts

to a small spherical body, rather smaller than a red blood corpuscle, but still containing pigment granules. The ookinete becomes enclosed in a cyst wall and grows to a size of $50\ \mu$ to $60\ \mu$ in diameter, the nucleus dividing into numerous small particles and the cytoplasm becoming vacuolated. Finger-like processes are formed on the surface of the cytoplasm and into each of these a nucleus passes to form the sporozoite. The sporozoites break away from their attachments and form a tangled mass in the oocyst, which also contains pigmented residual bodies. The number of oocysts present in one mosquito varies from one or two to forty or more. The oocysts burst and the sporozoites migrate through the body cavity to the salivary glands, where they remain infective until about the fiftieth to the sixtieth day after the mosquito has become infected. The duration of the development of *Plasmodium* in the mosquito differs according to the species of parasite and of vector and according to the temperature of the environment. Development does not appear to occur at temperatures below 16°C .

SPECIES OF PLASMODIUM AFFECTING MAN

Plasmodium vivax (Benign tertian malaria)

The developing trophozoites in the erythrocytes assume a signet-ring form. They are about $3\ \mu$ across, *i.e.* about one-third the diameter of a red blood corpuscle. The invaded cells increase in size, becoming slightly paler and after about 30 hours' growth, a number of fine granules (Schüffner's dots), which stain red with Romanowsky stains, appear. Typically, schizogony results in the production of 16 nuclei which fill the cell, which enlarges to about $11\ \mu$ in diameter. The febrile phase, associated with the release of the merozoites, occurs after every 48 hours. Gametocytes, the development of which takes place almost entirely in the spleen or bone marrow, appear about nine days after the appearance of parasites in the blood stream and resemble developing schizonts. The male cell is usually oval, about $7\ \mu$ to $8\ \mu$ across. The female cell is rather larger (about $10\ \mu$ in diameter) and is oval or round.

Plasmodium malariae (Quartan malaria)

The young forms (trophozoites) have a signet-ring appearance and are difficult to distinguish from *P. vivax*. As growth of the parasite proceeds, the organisms tend to be stretched as a band across the diameter of the corpuscle. The pigment formed is dark brown. Typical Schüffner's dots are not produced but a fine stippling (*Ziemann's dots*) may be seen. Six to ten merozoites are formed. Schizonts mature in 72 hours so that attacks of malaria occur every fourth day.

P. ovale (Ovale tertian malaria)

This parasite resembles *P. vivax* but induces only slight enlargement of the host-cell. Schüffner's dots are numerous but they lack the definition of those in *P. vivax*. The young trophozoites are compact and the chromatin nucleus is large. The usual number of merozoites does not exceed 8. The gametocytes are smaller than those of *P. vivax* and have golden brown pigment. There is a 48-hour periodicity of the clinical disease.

P. falciparum (Subtertian (malignant) malaria)

With this species the trophozoites are very small, occupying only about one-sixth of the diameter of the corpuscle, which is not enlarged. Multiple infections are common, and there is a tendency for the parasite to adhere to the edge of the corpuscle. The pigment is jet black. Irregularly shaped inclusions known as *Maure's dots* are formed. Clumping of infected cells occurs in the blood stream. Development of the schizonts occurs mainly in the internal capillaries, particularly those of the brain. Merozoites are small ($0.75\ \mu$ to $0.8\ \mu$) and number about 32. Schizogony proceeds irregularly and the cycle may be 48 hours or longer. Gametocytes are crescentic and are one and a half times as long as the red blood corpuscle. They are produced in cells in the internal organs and do not appear in the circulation until the infection has lasted for two to three weeks.

MALARIAL PARASITES OF BIRDS

PLASMODIUM GALLINACEUM Brumpt, 1935

P. gallinaceum has been used extensively in the laboratory for work relating to human malaria. The parasite has been

recorded from fowls in Ceylon, Indo-China, Malaya and Sumatra. Natural outbreaks in fowls have been described by Crawford (1945). Usually, indigenous stock is resistant to the pathogenic effects of the disease which, however, may cause heavy loss in imported stock. Young forms of the parasite may be as small as $1\ \mu$ in diameter and consist of a chromatin granule and cytoplasm. The mature schizonts may be as large as $8 \times 5\ \mu$ and contain from 8 to 30 merozoites. Exoerythrocytic schizogony occurs in bird malaria as in human malaria. There may be multiple invasion of a corpuscle even with schizonts. The gametocytes are spherical, about $8\ \mu$ in diameter, and displace the nucleus of the host-cell. The microgametocytes stain blue with Giemsa, the pigment granules being collected into a mass, whilst the macrogametocytes stain rose pink, the pigment granules being scattered through the protoplasm. The infection is transmissible by blood inoculation and Brumpt (1936) has shown that it can be transmitted cyclically by *Stegomyia fasciata* and *S. albopicta*.

Symptoms and pathology

In fowls, the incubation time after infection is five to ten days. Das (1952) noted an average of seven days using an indigenous

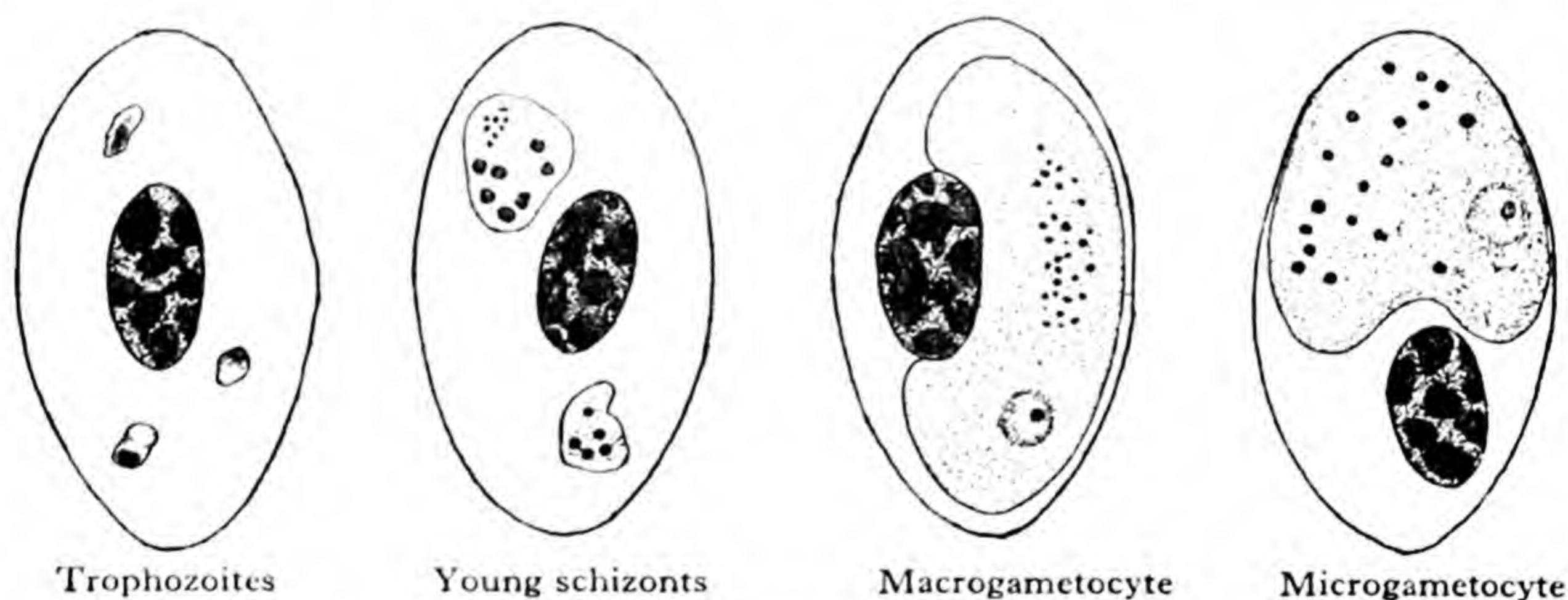


FIG. 23.—*Plasmodium gallinaceum* in erythrocytes of fowl.

strain in India. The parasites are numerous in the blood for seven to twenty-seven days depending on the severity of the attack. As many as 90 per cent. of the corpuscles may be invaded. There is a slight rise of temperature for a few days, when the parasites first appear in the peripheral blood. After this the temperature returns to normal if recovery is to follow, or becomes sub-normal if the prognosis is unfavourable. Birds become

unthrifty as the disease advances, then either die or start to recover and recommence feeding. Clinical anæmia is marked at the peak of the infection as judged by the pallor of the comb. There may be a greenish diarrhœa. Mortality varies greatly, adult birds imported into infected areas sometimes suffering a mortality of up to 80 per cent., while local birds and imported chicks may contract a mild disease with little or no mortality. When death occurs, it is usually associated with the presence of exoerythrocytic forms, which are not susceptible to treatment with quinine.

At post-mortem examination the carcase is invariably emaciated, with the spleen enlarged as much as six times its normal size and of a characteristically slatey-grey colour. The liver is usually dark-grey. There is an effusion of the pericardial sac with a jelly-like fluid. There is little abnormality in the brain and heart except for capillary congestion (Das, 1952).

Chemotherapy

Das (1952) reports Paludrine as being highly efficacious at a daily oral dose of 0.075 gm. per kilo body weight, for three successive days. Crawford (1945) in Ceylon obtained good results with 5 grains of quinine daily for seven days, together with Glauber's salts in the drinking water.

Under experimental conditions, various workers have found that quinine, Mepacrine and Amodiaquin did not protect fowls from artificial infection with the sporozoites of *P. gallinaceum*. Chloroquin (5 mg./kilo) suppressed infection for at least a month. Pamaquin and Primaquine (especially) were both effective. Sulphadiazine (100 mg./kilo), Proguanil (7.5 mg./kilo) and Bromoguanidine (15 mg./kilo) afforded full protection from infection. Pyrimethamine was effective at a dose of 0.3 mg./kilo.

Immunity

Untreated birds are usually solidly immune. Treated birds may remain susceptible.

MALARIA IN TURKEYS

Malaria of turkeys has been described in Russia, the parasite being named *P. malarie raupachi* Parcvanidze, 1914. In Kenya, a similar parasite has been described as *P. duræ* Herman, 1941.

It is suggested that the Kenya form is a malaria of wild birds to which turkeys are susceptible and that it may differ from the Russian form. The parasite is definitely pathogenic in natural outbreaks in Kenya. Herman (1941) described the trophozoites as possessing a single pigment granule and a refractile globule. The presegmentation stage frequently formed a rosette or ring and tended to assume a polar position in the erythrocyte. The number of merozoites varied from 6 to 14, usually being 8. The gametocytes were elongated, the pigment granules being round and black. After intravenous inoculation, the prepatent period varied from three days to two and a half weeks. The infection was rapidly fatal. Chickens, pigeons and canaries could not be infected by inoculation.

MALARIA IN PASSERINE BIRDS

Malaria in the canary has been studied particularly with a view to possible relationships with human malaria. A number of species has been described of which *P. relictum* and *P. cathe-merium* have particularly been used for experimental work.

MALARIA IN WATER BUFFALO

P. bubalis has been recorded in India and Indo-China. The parasite resembles *P. malariae*. Young ring forms are said to measure $1.5\ \mu$ to $2\ \mu$ in diameter and adult trophozoites 5 to $6\ \mu$, whilst the female gametocyte is about $6\ \mu$ in diameter. According to Rao (1938) infection is cryptic and becomes apparent only under the influence of intercurrent disease. Out of eleven cases, one showed hæmoglobinæmia.

MALARIA IN MONKEYS

A number of species has been recognised and been used for research into the problems of human malaria. *P. reichenowi* resembles *P. falciparum* and *P. kochi* resembles *P. vivax*. *P. knowlesi* occurs naturally in *Macaca irus* and, experimentally, infects man. *P. cynomolgi* in the monkey fed on a milk diet develops only a low-grade infection. Both *P. cynomolgi* and *P. knowlesi* are believed to resemble the human species closely in their reaction to drugs.

OTHER SPECIES

P. berghei, Vincke and Lips (1948) was found in the tree rat (*Thamnomys surdaster*) of the Congo and can be transferred, in the laboratory, to white mice, white rats and cotton rats. It has been used to a considerable extent for experimental work in the laboratory. It appears, however, that the response of *P. berghei* to drugs is often different from the human and other species of malaria. In particular it is extremely sensitive to sulphadiazine and rather less sensitive to proguanil.

CHEMOTHERAPY OF MALARIA

An immense literature has accumulated on this subject. For general information the reader is referred to such text-books as

TABLE XII. SOME SPECIES OF MALARIAL PARASITES

Species	Vertebrate host	Species	Vertebrate host
<i>Plasmodium vivax</i> . (Grassi and Feletti, 1890)	Man	<i>P. brazilianum</i> . Gonder and Gossler, 1908	Monkey
<i>P. falciparum</i> . . . (Welch, 1897)	Man	<i>P. bubalis</i> Sheather, 1919	Water buffalo
<i>P. malariae</i> (Laveran, 1881)	Man	<i>P. gallinaceum</i> . . . Brumpt, 1935	Fowl
<i>P. ovale</i> Stephens, 1922	Man	<i>P. duræ</i> Herman, 1941	Turkey
<i>P. reichenowi</i> . . . Sleiter <i>et al.</i> , 1922	Monkey	<i>P. malariae raupachi</i> . Parkvanidze, 1914	Turkey
<i>P. kochi</i> (Laveran, 1899)	Monkey	<i>P. relictum</i> (Grassi and Feletti, 1891)	Passerine birds, Finches, Canaries, etc.
<i>P. inui</i> Halberstädte and Prowazek, 1907	Monkey	<i>P. cathemerium</i> . . . Hartman, 1924	
<i>P. semnopithecii</i> . . Knowles, 1919	Monkey	<i>P. rouxi</i> Sergent and Catanei, 1928	
<i>P. pitheci</i> Halberstädte and Prowazek, 1907	Monkey	<i>P. elongatum</i> Huff, 1930	Rodents
		<i>P. berghei</i> Vincke and Lips, 1948	

Text-book of Pharmacology (Salter, 1952). More recent advances have been reviewed by Goodwin and Rollo (1955). The various drugs used in the treatment of malaria differ in their effects on the

different developmental stages of the parasites. Different species of *Plasmodium* may, moreover, differ to some extent in their reactions to the different drugs. The following summarises the therapeutic agents and their usual effects :—

(1) **The Cinchona Group** (*e.g.* Quinine, Totaquinine and related substances)

These are the oldest known therapeutic agents. They have a marked effect on the erythrocytic asexual forms, *e.g.* the trophozoites and the schizonts.

(2) **The 9-Aminoacridines** (Quinacrine, Atabrine, Mepacrine)

These affect the erythrocytic asexual stages.

(3) **The 4-Aminoquinolines** (Chloroquine, Amodiaquin)

These act similarly to the 9-Aminoacridines but appear to be more active, do not stain the skin and do not cause such marked intestinal disturbance. They act more rapidly in acute malaria than does Mepacrine.

(4) **The 8-Aminoquinolines** (Pamaquin, Pentaquin, Isopentaquin)

These are used particularly as an adjunct to Quinacrine. By themselves they are useless as suppressants (having a very weak effect on the erythrocytic schizonts) but they affect the pre-erythrocytic stages and inhibit the development of the exoerythrocytic forms. They have a marked effect on the gametocytes.

(5) **The Biguanides** (Proguanil, Chloroguanide, Paludrine)

These prevent the development of the exoerythrocytic forms and they affect also the pre-erythrocytic and the asexual erythrocytic forms. Chloroguanide does not kill the gametocytes but the parasites are prevented from developing normally in the mosquito.

(6) **The Sulphonamides** (Sulphapyrazine and Sulphamerazine)

With some species of malaria these have a suppressive effect on the asexual erythrocytic forms. Sulphadiazine is a prophylactic against *P. gallinaceum* in chicks and has been shown to inhibit the development of sporozoites in the vector. No such inhibition is, however, observed in the development of the sporozoites of *P. falciparum* of man.

(7) **The Pyrimidine series** (Daraprin (pyrimethamine), Malocide)

2, 4, diaminopyrimidines have been shown to be highly active as suppressants of malaria. Their use in synergistic combination with sulphonamides has been described by Rollo (1955). Pyrimethamine has an effect on the sporozoites and the tissue forms of human malaria and also some effect on the gametocytes.

CHAPTER XI

THE FAMILY BABESIIDÆ Poche, 1913

SPECIES OF BABESIA : CONTROL OF BABESIOSIS

IN this Family are included the parasites which inhabit the red blood corpuscles of mammals, but which do not form the pigment which is so characteristic of the members of the *Plasmodiidæ*.

The systematics of the group have presented considerable difficulties, largely owing to the extremely small size of many of the parasites and the incomplete knowledge of their life-histories. Kudo (1954) recognises the genera *Babesia*, *Theileria* and *Toxoplasma* but makes no reference to *Ægyptianella* which can hardly be separated from *Babesia*.

Many authors divide the genus *Babesia* into three sub-genera which are differentiated according to the number of divisions which occur in the erythrocytes. In the sub-genus *Babesia*, the parasite divides once, so that each corpuscle is likely to contain two parasites. In *Nuttallia* two divisions result in the occurrence of four parasites, while in *Ægyptianella* more than four divided forms occur.

Some authors divide the genus *Babesia* into *Babesia* and *Babesiella*. Tsur, Hadani and Pipano (1960) have described an exoerythrocytic schizogony in a piroplasm of gerbils (*Meriones tristrami shawii*). They named the parasite, which reproduced also in the red blood cells, *Nuttallia danii*.

Genus *BABESIA* Starcovici, 1893

General characters and morphology

The organisms are non-pigmented amœboid parasites of the red blood corpuscles. They reproduce within the corpuscle by division into pear-shaped forms which are arranged in couples, often at a characteristic angle, one to the other, with the narrow ends apposed. Stained with Romanowsky stains, the organisms show a blue cytoplasm and a red chromatin mass from which project strings of chromatin granules. This represents the nucleus.

In many domestic animals at least two species of piroplasm are found, one being large and the other small, when in the red blood corpuscle. This differentiation has allowed a convenient division into two groups which are separated, as a rule, by a different response to drugs. Thus, in cattle the large piroplasm *B. bigemina* may be several microns in length and extend across the host corpuscle. By contrast, *B. bovis* is only about $1.5\ \mu$ long. *B. bigemina* is suppressed by trypan blue which has, however, no effect on *B. bovis*.

Life-cycle of the parasite

This has been followed most fully in *Babesia canis* of the dog and in *B. bigemina* of cattle.

As shown in the classic work of Smith and Kilborne (1893), part of the life cycle is spent in a tick. Cattle infected with *Babesia bigemina* contain in their erythrocytes oval or piriform bodies with a compact nucleus and vacuolated cytoplasm. Multiplication of these bodies takes place by a characteristic budding process which is probably similar to the schizogony shown by other members of the Sporozoa.

From observations in experimentally infected calves Getta (1957) concluded that *B. bigemina* and *Francaiella cochlica* (*B. bovis*?) first multiplied in capillaries of the internal organs, the greatest proportion of infected erythrocytes being seen in smears of heart muscle, kidney, adrenal gland and brain. In some species of *Babesia*, e.g. in *B. muris* of the white rat and in *B. ovis* of the sheep multiplication by schizogony in the cells of the spleen and the kidneys has been described. As the result of multiplication, two or more organisms may occur in each cell. The affected blood cell disintegrates, setting free the contained piroplasms which then invade other corpuscles and again grow into amœboid forms and multiply. Infection may be effected by the mechanical transfer of blood from one animal to another.

When, however, infected blood is taken up by a tick of suitable species a complicated type of development has been described by Dennis (1932). When the infected blood has been taken into the gut lumen of the tick, *isogametes*, that is gametes which do not appear to differ in morphology, are produced and fusion results in the production of motile club-shaped zygotes (*ookinetes*).

These pass through the gut wall and invade the ova of the tick. Each parasite rounds off to form a sporont which grows in size. Multinucleated amœboid *sporokinetes* result from the repeated division of the nucleus of the sporont. These migrate through the embryonic tissue cells of the tick to the salivary glands, eventually developing into sporozoites which infect fresh cattle when the tick begins to feed. Thus, typically, infection passes through the egg and transmission to a new host is effected by ticks of the next generation. Brumpt (1937) reported that infection with *Babesia* may persist through five generations of ticks which have fed on non-susceptible hosts.

Other accounts of the development of *Babesia* in ticks have been recorded by Kapustin (1955), Petrov (1941), Regendanz and Reichenow (1933), and Shortt (1936). Some authorities do not consider that sexual reproduction occurs in *Babesia*.

Species of animal affected

Neitz and Steyn (1947) discuss the susceptibility of various animals to *Babesia* infection and conclude that they are by no means as host-specific as formerly believed. They conclude, for instance, that *B. bigemina* parasitises the white-tailed deer as well as cattle in the Americas, that *Nuttallia (Babesia) equi* is found in horse, mule, donkey and zebra and that *Babesia canis* and *B. gibsoni* are both found in carnivores other than the dog. Susceptibility to infection with *Babesia* often increases with age. Young animals may be resistant. Susceptibility can usually be increased by the removal of the spleen.

Pathogenicity

Young animals often contract symptomless infection with *Babesia* and in an area of endemic *B. bovis*, for example, the presence of the parasite may not be suspected until susceptible adults are introduced. In general, *B. bovis* can be regarded as a relatively benign parasite while *B. bigemina* is considerably more pathogenic and can cause heavy mortality. Canine piroplasmiasis is usually a severe disease, particularly with dogs imported into the tropics as adults.




TABLE XIII. SOME SPECIES OF BABESIIDÆ

Host	Species	Transmitting tick	Stages at which infection is transmitted
LARGE FORMS			
Dog	<i>B. canis</i> (Piana and Galli-Valerio, 1895)	<i>Dermacentor</i> (3 species) Russia and Europe <i>Hæmaphysalis leachi</i> (South Africa) <i>Hyalomma marginatum</i> Russia <i>Rhipicephalus sanguineus</i> various places	Nymph and adult Adult Adult Nymph and adult
Horse	<i>B. caballi</i> (Nuttall and Strickland, 1910)	<i>Dermacentor</i> (3 species) Europe and Russia <i>Hyalomma</i> (4 species) Europe, North Africa and Russia <i>Rhipicephalus</i> (2 species) Europe	Nymph and adult Nymph and adult Nymph and adult
Ox	<i>B. bigemina</i> (Smith and Kilborne, 1893)	<i>Boophilus</i> (several species) North and South America, Australia, Panama, Africa <i>Hæmaphysalis punctata</i> Europe <i>Rhipicephalus</i> sp. Africa	Larva Adult Larva, Nymph, Adult
Sheep	<i>B. motasi</i> Wenyon, 1926	<i>Rhipicephalus bursa</i> Europe	Adult
Swine	<i>B. trautmanni</i> Knuth and du Toit, 1921	<i>Rhipicephalus</i> sp. Europe <i>Hyalomma</i> sp. Europe <i>Dermacentor</i> sp. Europe <i>Boophilus</i> (?) East Africa	Adult ? ? ?

SMALL FORMS

Dog	<i>B. gibsoni</i> (Patton, 1910)	<i>Hæmaphysalis bispinosa</i> India	Larva, Nymph, Adult
		<i>Rhipicephalus sanguineus</i> India	Nymph and adult
Ox	<i>B. major</i> Sergent <i>et al.</i> , 1926	not known	
	<i>B. argentina</i> (Lignières, 1901)	<i>Boophilus microplus</i> Argentina and Australia	Larva
	<i>B. bovis</i> (Starcovici, 1893)	<i>Ixodes ricinus</i> Europe	Larva, Nymph, Adult
		<i>Ixodes persulcatus</i> Russia	Larva, Nymph
	<i>B. divergens</i> (M'Fadyean and Stockman, 1911)	<i>Ixodes ricinus</i>	Larva, Nymph(?)
Sheep	<i>B. ovis</i> (Starcovici, 1893)	<i>Rhipicephalus bursa</i> Russia	Adult
Cat	<i>B. felis</i> (Carpano, 1934)	not known	
Swine	<i>B. perroncitoi</i> Cerruti, 1939	not known	
Horse	<i>B. (Nuttallia) equi</i> Laveran 1901	<i>Dermacentor</i> (2 species) Europe and Russia	Adult
		<i>Hyalomma</i> (4 species) Europe, Asia, Africa	Adult
		<i>Rhipicephalus</i> (3 species) Russia, Asia, Africa	Adult
Cat	<i>B. (Nuttallia) felis</i> (Davis, 1929)	not known	
Fowl	<i>Ægyptionella pullorum</i> Carpano, 1928	<i>Argas persicus</i>	

Symptoms and pathology

The incubation period after infection varies between five and about ten days. The parasites destroy the red cells with a release of hæmoglobin. If the destruction is limited, the body is able to dispose of the breakdown products but anæmia of varying degree will be observed. As the parasites increase in numbers, the liver



FIG. 24.—Cycle of *Babesia canis* transmitted by *R. sanguineus*.

1. Female takes up infected blood-sporoblasts from in subcutis.
2. Gravid female-sporoblasts migrate to ovaries and parasitize ova.
- 3, 4, 5. Next generation larvæ, nymphs and imago with infested salivary glands.
6. Sporozoites inoculated into blood stream of dog. Invade erythrocytes.
7. Multiplication by budding in canine erythrocytes.

and kidneys become involved. The kidneys become blocked, the tubules degenerate and hæmoglobin is excreted with the urine. The liver is unable to dispose of the excess bile formed ; there is a parenchymatous degeneration, plugging of the bile ducts and resultant jaundice. The spleen is enlarged owing to engorgement with red blood cells and may rupture. The pulp appears dark and the splenic corpuscles are prominent. The stomach and small intestine show catarrhal gastro-enteritis. Characteristically,

there is an acute phase to the disease, corresponding with the appearance of a maximum concentration of the parasites in the peripheral circulation. This usually occurs a few days after infection. Thereafter, the urine may become red to black in colour according to the severity of erythrocyte destruction. Death may occur at this stage.

The number of parasites in the circulation soon decreases. If the animal survives the initial phase it may nevertheless die two or three weeks later, showing general symptoms of anæmia and debility. Cerebral piroplasmosis, as described by Tchernomoretz (1943) and Zlotnik (1953) in cattle, and by Purchase (1947), in the dog, can induce a rapid and extremely fatal disease.

PIROPLASMOSIS IN THE DOG

Two principal species are recognised—*Babesia canis* and *Babesia gibsoni*.

BABESIA CANIS (Piana and Galli-Valerio, 1895)

Morphology

This is one of the large piroplasms, with the characteristics of the genus, typically pear-shaped but varying according to the state of growth and in stained preparations according to the fixation. The organism is $4.5\ \mu$ to $5\ \mu$ in length, pointed at one end and round and bulbous at the other. There is generally a vacuole in the cytoplasm. Often multiple infection of the corpuscles is observed.

Distribution and transmission

Africa, Asia and Southern Europe and in some limited areas in the U.S.A. Transmission is by species of *Rhipicephalus*, *Dermacentor*, *Hyalomma* and *Hæmaphysalis*. Stage to stage transmission can occur.

Species susceptible

Dog, Jackal, Wolf.

Symptoms and pathogenicity

In the tropics the disease is known as biliary fever or tick fever of dogs. Both young and old dogs appear to be susceptible

and there is a particularly high mortality among dogs imported from Europe. In acute cases there is high fever rapidly followed by a very marked anæmia with jaundice. This is followed by

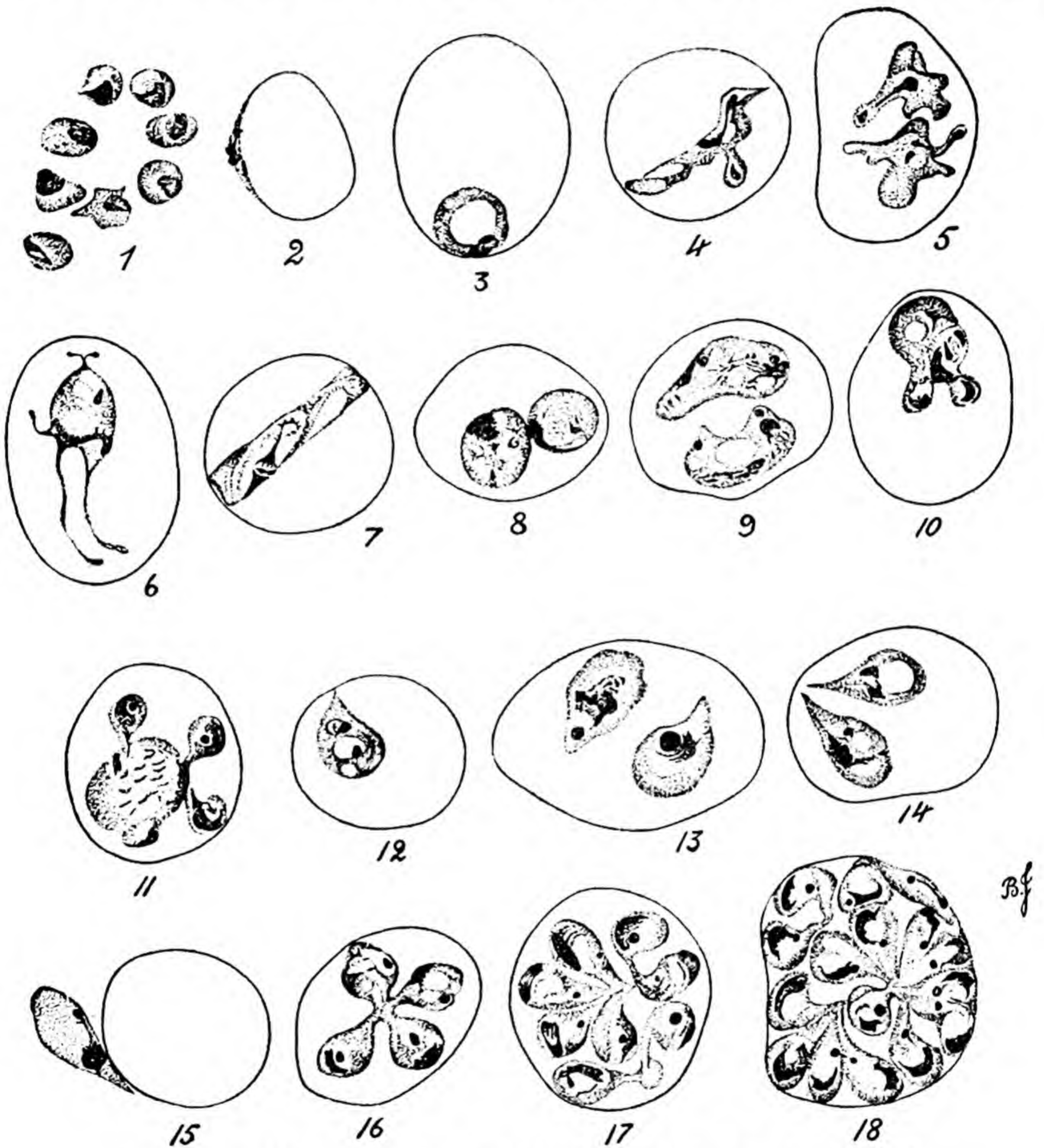


FIG. 25.—*Babesia canis* in the blood of a dog. (\times about 2250)

1. Groups of free forms probably resulting from rupture of a cell with multiple infection. 2. Marginal form. 3-9. Various types of parasite. 10. Form producing two buds. 12-14. Pear-shaped forms. 15. Free pear-shaped forms. 16. Form producing four buds. 17 and 18. Cells containing several buddings.

(from Wenyon, 1926. After Kinoshita, 1907)

collapse, often quite sudden in onset, with a sub-normal temperature and death. In indigenous dogs recovery is common but may take several weeks.

Purchase (1947) has described the condition of cerebral piroplasmosis in the dog. A dog was brought for inspection when in a comatose state and with a temperature of 102° F. Parasites were rare in the blood but very numerous in the brain. In brain sections the parasites were seen to be filling the lumen of the small capillaries and arterioles where they were nearly all extra-cellular lying free in the vessels.

Diagnosis

In areas where the disease is known to be endemic any dog with a high temperature should be examined for parasites which if present in the peripheral blood can readily be demonstrated with Giemsa or Lieshman stains. If the history suggests that a high temperature has preceded examination and if there is a marked anæmia then the dog is best treated as a case of babesiosis, even when microscopic examination of the blood fails to detect parasites. In chronic cases the presence of the parasite may sometimes be demonstrated by the sub-inoculation of blood to a susceptible (preferably splenectomised) dog. It must be stressed that actual demonstration of parasites in the peripheral blood of field cases may require prolonged search.

Treatment

A single intravenous injection of 4 to 5 ml. of a 1 per cent. solution of trypan blue is usually sufficient for an average-sized dog (35 lb.) and there is rapid recovery. Great care must, however, be taken to ensure that none of the solution gets outside the vein. Extensive sloughing is otherwise liable to occur. This sloughing rarely fails to heal but causes a great deal of unnecessary suffering to the dog when already in a weak condition. Solutions should be freshly prepared, filtered before use and diluted as far as convenient having regard to the size of the animal. Acaprine and its analogues are similarly very effective and are safer to use under practical conditions. They are given subcutaneously. Particularly in India, acriflavine has been used very successfully. Phenamidine, given at the rate of 10 mgm. per kgm. body weight subcutaneously has given excellent results.

Immunity

It is probable that animals never acquire a sterile immunity, either naturally or as the result of treatment. In those instances where it has been possible to follow the course of the parasitæmia in untreated animals it has been shown that the parasites are not present to any extent in the peripheral circulation for more than a few days. Untreated cases which survive speedily acquire

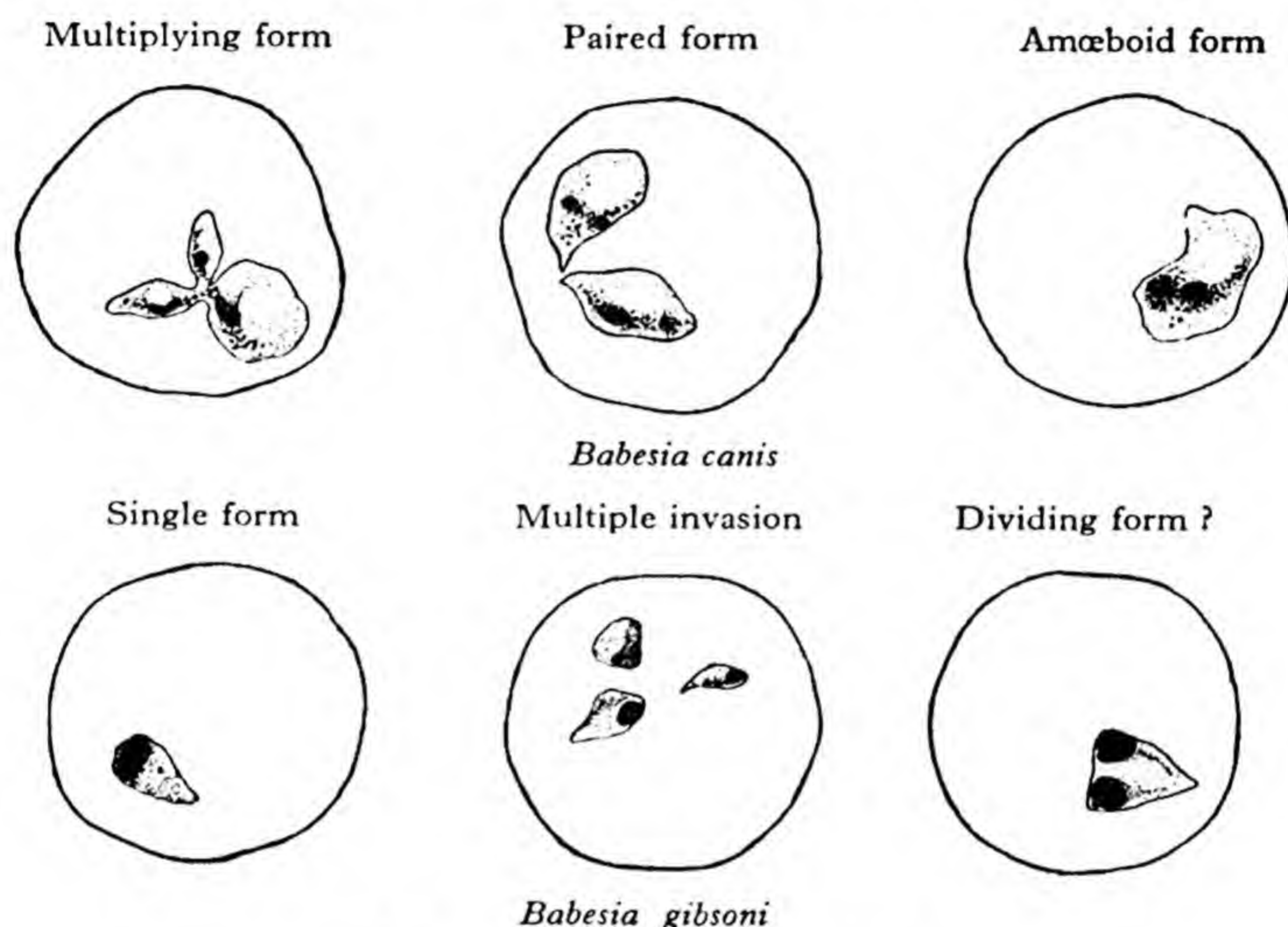


FIG. 26.—Canine piroplasms.

a premune state and it is probable that the effect of therapy is to tide the animal over the acute stage of the disease while permitting a state of premunity to arise. It is usual in endemic areas to find the majority of the dog population harbouring the parasite. Relapse of such animals to clinical disease classically follows such stress factors as intercurrent disease, or, under laboratory conditions, splenectomy.

BABESIA GIBSONI (Patton, 1910)

This parasite has been found in the dog, jackal, wolf and fox. It occurs in India, Ceylon and probably much of the Far East. It has occasionally been reported from Africa. It differs from *B. canis* in being pleomorphic. Most forms are smaller than *B. canis*. Typically, signet-ring forms, large circular blue rings with

chromatin dots, will be seen in lightly stained smears. There may be small rod-shaped parasites among others which bear a more marked resemblance to *B. canis*. Transmission is by species of *Rhipicephalus* and *Hæmaphysalis*.

The disease produced by *B. gibsoni* is usually more chronic than that attributed to *B. canis*. There are remissions and exacerbations of the febrile condition with increasing anæmia. Death may take place after many months' illness. The principal signs of disease are profound anæmia, highly coloured urine, constipation and very marked enlargement of the spleen and liver. Jaundice seldom or never occurs as the result of infection with *B. gibsoni*.

B. gibsoni differs markedly from *B. canis* in the failure to respond to trypan blue or Acaprine. Symptomatic treatment with arsenicals is reported to be of some value.

BABESIA BIGEMINA (Smith and Kilborne, 1893)

This is the causal organism of cattle tick fever (red water disease) in many parts of the world. In the U.S.A. the disease, which was formerly very prevalent, is known as *Texas fever*.

Morphology

B. bigemina is one of the "large" piroplasms, measuring $4\ \mu$ - $5\ \mu$ in length by $2\ \mu$ wide. In the red blood cells of cattle the organism may assume various shapes but the most characteristic is oval to pear-shaped. Paired parasites usually appear to form an acute angle in the corpuscle.

Distribution

The parasite occurs throughout the tropics and in many subtropical countries including parts of Australia and Southern Europe, including Hungary (Kotlán, *et al.*, 1959).

Transmission and life-cycle

Smith and Kilborne (1893) first investigated the nature of Texas fever and associated the disease with the presence of the tick. Dennis (1932) investigated the life-cycle in *Margaropus annulatus* and reported that there was an asexual cycle in the vertebrate host and a sexual cycle in the tick. Transmission of

B. bigemina is through species of *Boophilus* or *Margaropus* which many regard as a synonym for *Boophilus*, and by species of *Rhipicephalus* and *Hæmaphysalis*.

Symptoms and pathogenicity

The incubation period is usually one to two weeks. The typical symptoms are fever (lasting for two to three days), anæmia, hæmoglobinuria, cardiac palpitation and a characteristic series of digestive disturbances (diarrhœa followed by constipation). The mortality may be very high during the acute febrile phase when the rapid breakdown of red blood corpuscles leads to hæmoglobinuria, and also following chronic disease which may extend over several weeks with an irregular course and intermittent temperatures of 104° F. to 105° F. Some recent workers (Zlotnik, 1953) have called attention to cerebral piroplasmosis in cattle. This condition simulates heart water disease. As seen in Nyasaland, the onset of the disease is sudden and in a few hours a temperature of 106° F. to 107° F. may be recorded with death in 12 to 36 hours. The blood picture shows little alteration and the organisms are absent or very rarely seen in blood smears. The parasites accumulate and most probably multiply in the cerebral capillaries only.

Diagnosis

Diagnosis is made on the presence of parasites in the peripheral circulation and on the characteristic appearance of hæmoglobinuria, but as discussed under *B. bovis* the classic picture of the disease is by no means always apparent and it may be necessary to examine a number of smears in order to establish the presence of the parasite. In endemic areas a spot diagnosis can often be made on the appearance of animals with a high temperature and a typical stiffness of gait.

Treatment

The disease responds well to trypan blue, about 100 ml. of a 1 per cent. solution being given in normal saline intravenously with the usual precautions to ensure that none of the solution is injected subcutaneously. Acaprin (Bayer) and other related substances can be given subcutaneously. Trypaflavine (acri-flavine 0.5 gm. to 1.0 gm.) may be given intravenously as a 1 in 100

solution. Phenamidine (May and Baker) has proved highly effective.

Immunity

Young stock, up to a year in age, is highly resistant to the parasite and may be immunised by the injection of blood from carrier animals. If it is considered necessary to immunise adult stock, the disease must be controlled therapeutically. Breakdown in the resistance of premune stock often seems to follow stress factors such as rinderpest immunisation.

Epidemiology

The disease most characteristically occurs following the movement of susceptible adult stock into an endemic area or following the movement of tick-infested carrier stock into a clean tick-free area. It is believed (Morgan and Hawkins, 1948) that in the U.S.A. small endemic foci are kept alive by the deer reservoir hosts of the vector ticks.

BABESIA BOVIS (Starcovici, 1893) and *BABESIA DIVERGENS* (M'Fadyean and Stockman, 1911)

Simitch, Petrovich and Rakovec (1955) and Davies, Joyner and Kendall (1958) investigated the status of the small piroplasm found typically in Northern Europe (including Britain) and have concluded that it differs from the species originally found in the Danube basin and described by Babes. This latter species remains *B. bovis* while the north European form becomes *B. divergens* (M'Fadyean and Stockman). *B. bovis* is characteristically found in Southern Europe but has been reported also from Africa, Asia and the East Indies. It occurs mainly outside the tropical zones. It is quite clear that for many years there must have been confusion in the literature between the two species and the following account should be read with this in mind.

Morphology

Both species belong to the group of "small" piroplasms. In blood smears taken at the height of the parasitaemia *B. divergens* appears as paired, divergent, club-shaped organisms measuring about $1.5 \times 0.4 \mu$ and lying superficially in the corpuscle. There may be occasional stouter forms up to $2.0 \times 1.0 \mu$. *B. bovis*, by

contrast, is on average larger, typical individuals measuring $2.4 \times 1.5 \mu$ and there are no divergent forms lying superficially in the corpuscle. Nearly all the parasites are vacuolated "signet-ring" forms consisting of a centrally placed vacuole with nuclear granules concentrated at one point on the periphery of the circular zone of cytoplasm.

Life-history and transmission

Little is known about the development of either species. *B. divergens* is certainly transmitted by *Ixodes ricinus* and observations on the infection have been made under experimental conditions by Davies, Joyner and Kendall (1958) but as far as is known the development within the tick has never been recorded. As regards *B. bovis*, *Ixodes ricinus* may be a vector. Petrov (1941) reported that parasites had been identified in the gut wall, ovary and egg of *Ixodes ricinus* and in various parts of the body and particularly the salivary glands of the nymph.

Garnham and Bray (1959) after the apparently authentic report of a case of *Babesia* infection in man were able to show that the British species of *Babesia* (*B. divergens*) could be transmitted to splenectomised chimpanzees and that a fulminating infection might result.

Symptoms and Pathology

The clinical picture is probably similar in the two species.

Wright and Woodford (1958) give an account of the clinical disease and its epidemiology in Britain.

The disease is not usually as severe as that associated with *B. bigemina*. The incubation period varies from about four to ten days, the first symptom being the appearance of a high temperature (104° F. to 106° F.) which usually lasts from two to three days only. Hæmoglobinuria may be noted within about 48 hours of the appearance of the high temperature. Rapidly fatal cases with fulminating infection are occasionally seen. If the animal survives the initial phase, diarrhœa precedes constipation, the fæces being bile-stained and the anal sphincter being in a state of increased tone. The mucous membranes are pallid and there is jaundice. The heart beats may be very much accentuated and otherwise quiet animals become uncontrollable.

The appetite fails and bowel stasis increases. Death may occur.

Post-mortem examination shows necrosis of the liver in the central zone of the lobules. Fatty degeneration may be apparent.

Diagnosis

It is important to note that parasites are rarely found with any ease except during the two or three days during which the animal shows a high temperature. A number of blood smears may have to be examined before parasites are found. Diagnosis is usually made, tentatively at least, on history and epidemiology, together with the occurrence of hæmoglobinuria.

Epidemiology

The incidence of infection can usually be related to the occurrence and activity of the vector. In Britain, spring, summer and autumn infections are experienced. Fulminating infections occur most often when fully susceptible adult cattle are moved to an endemic area. Initially there may be a number of deaths but this is soon followed by cases of milder type. With the milder cases the hæmoglobinuria resolves itself within about 36 hours and convalescence is established within two or three days.

Disease of irregular occurrence may arise as the result of breakdown in premunised animals following unusual stress such as calving or prolonged periods of inclement weather. Animals up to about one year in age are usually highly resistant to infection, clinical effects are rarely seen and the parasites disappear from the peripheral circulation very rapidly. Such animals may, however, act as carriers for considerable periods of time—at least two years—and scanty parasites may very occasionally be found in blood smears, this indicating the way in which clean ticks can become infected.

Resistance

This state of premunition—a low-grade chronic infection—associated with a considerable degree of resistance to reinfection, is very characteristic of infection with the Babesias. A solid resistance, with the complete absence of parasites, has not yet been demonstrated. There is no cross immunity between *B. bovis* and *B. bigemina* (Neitz, 1941).

Treatment

B. bovis and *B. divergens* differ markedly from *B. bigemina* in that they fail to respond to treatment with trypan blue. Drugs of the Acaprin series, phenamidine, and according to Neitz (1941)

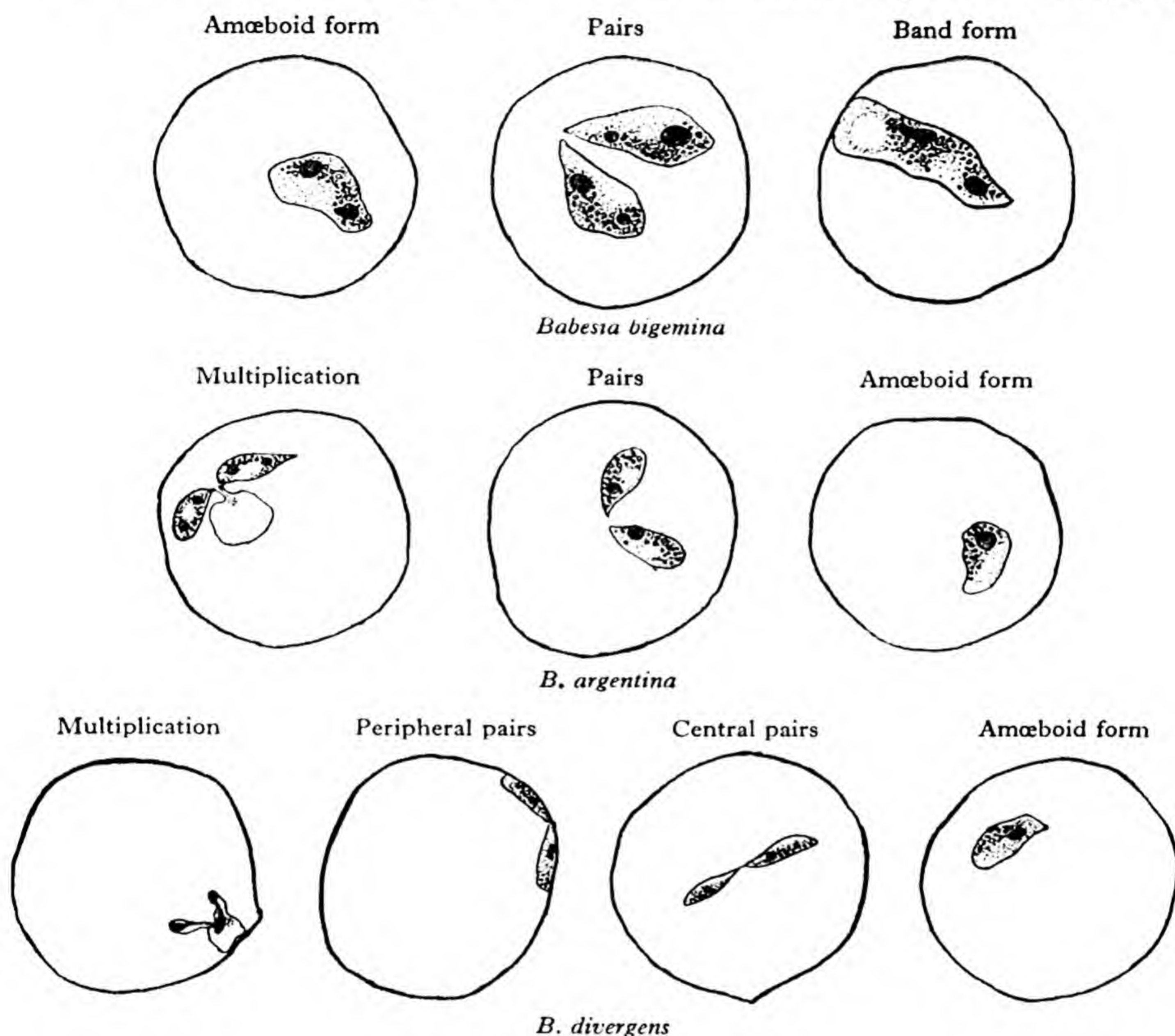


FIG. 27.—Piroplasms of ox.

Gonacrine (May and Baker) have all been reported to have a specific effect. In the field, however, experience shows that treatment late in the course of the disease (particularly when chronic constipation is evident) rarely has any effect. The effect of treatment in the initial acute phase of the disease, which in any event lasts only for a short time, is difficult to assess.

The course of the disease is definitely altered by symptomatic treatment and by careful nursing. For recent developments in chemotherapy see page 167.

OTHER SPECIES OF BABESIA IN CATTLE

A number of species has been described. Of these it is proposed to regard *B. argentina* (Lignières, 1901) as synonymous with *B. berbera* (Sergent *et al.*, 1924), and *B. major* as a separate species. Some authorities consider *B. argentina* to be synonymous with *B. bovis*. The species *Francaiella occidentale*, *F. caucasica* and *F. colchica* have been described in Russia but are almost certainly synonyms for previously described species.

B. ARGENTINA Lignières, 1901**Morphology**

The organism is more robust than the typical *B. bovis*, the pear-shaped forms measuring about $1.5\ \mu$ by $2.0\ \mu$ and being commonly found in the centre of the corpuscle. Occasional peripheral forms are found.

Distribution and transmission

Widely throughout the tropics, America, Africa and Asia and in the sub-tropics in South Europe and Australia. It is transmitted by species of *Boophilus* and (possibly) *Rhipicephalus*.

Pathogenicity

According to Seddon (1952), *B. argentina* in Australia causes a more virulent disease than *B. bigemina*. The parasites are comparatively rare in the peripheral blood but may be found fairly easily in smears from the heart or kidney, in which many parasites may be extra-cellular. Earlier Tchernomoretz (1943) had described *B. berbera* in Palestine as being less numerous than *B. bigemina* in the general circulation and showed that blocking of the brain capillaries was an important contributory cause of death. The parasites appeared to behave like the cerebral form of *B. bigemina*, both the cerebrum and the cerebellum being affected. Parasitised erythrocytes appear to adhere to the endothelium of the capillaries.

Immunity

Seddon (1952) reported that animals carrying a latent infection with *B. bigemina* showed resistance to infection with *B. argentina* but that carriers of *B. argentina* had no resistance to *B. bigemina*.

Hall (1960) has given evidence of the development of a passive immunity in young calves to infection with *B. argentina* as the result of the infection of their mothers during pregnancy.

BABESIA MAJOR Sargent *et al.*, 1926

This parasite has been reported from North, South and West Africa, Israel (Kemron, *et al.*, 1960), South America and from the Baltic region. In general it resembles *B. bigemina* but is smaller. It is distinguished morphologically from *B. bovis* by its elongated subpiriform shape, by the fact that most paired forms meet at an angle less than 90° , by its greater size and by its position in the centre of the erythrocyte. Sargent *et al.* (1945) stated that Ichthargan was the most effective drug.

Bool, *et al.* (1961) have reported the presence of a parasite resembling *B. major* in Holland where it may be transmitted by *Hæmaphysalis cinnabarina punctata*.

PIROPLASMOSIS IN EQUINES

Two species of *Babesia* are recognised in the horse: *B. caballi* (Nuttall and Strickland, 1910) and *B. (Nuttallia) equi*. Laveran, 1901.

BABESIA CABALLI (Nuttall and Strickland, 1910)

Morphology

This is one of the large type of parasites, resembling *B. bigemina* of cattle, the parasites commonly occurring as pear-shaped organisms lying in the corpuscles in pairs.

Distribution

South Europe through Asia, Russia, Africa and the Panama Zone. Transmission is by *Dermacentor*, *Hyalomma* and *Rhipicephalus*.

Kartashev (1957) reports that clavate forms of *Babesia caballi* were identified in eggs from two species of *Dermacentor* ticks. This was in an area where the disease was endemic in horses. Tsaprun (1957) found developmental stages in the salivary glands of *D. marginatus* and schizonts in the intestines of the tick.

Pathogenicity

Persistent fever, anæmia and, in severe cases, paralysis of the hind limbs are noted as the result of infection with *B. caballi*. Hæmoglobinuria is not characteristic.

Treatment

Trypan blue, Acaprin and acriflavine (Gonacrine), have all been reported as being effective against *B. caballi*.

B. (NUTTALLIA) EQUI Laveran, 1901

Morphology

The parasite is readily distinguished from *B. caballi* by its small size (barely $2.0\ \mu$ long) and by the characteristic division into four-daughter organisms which form a cross in the corpuscle.

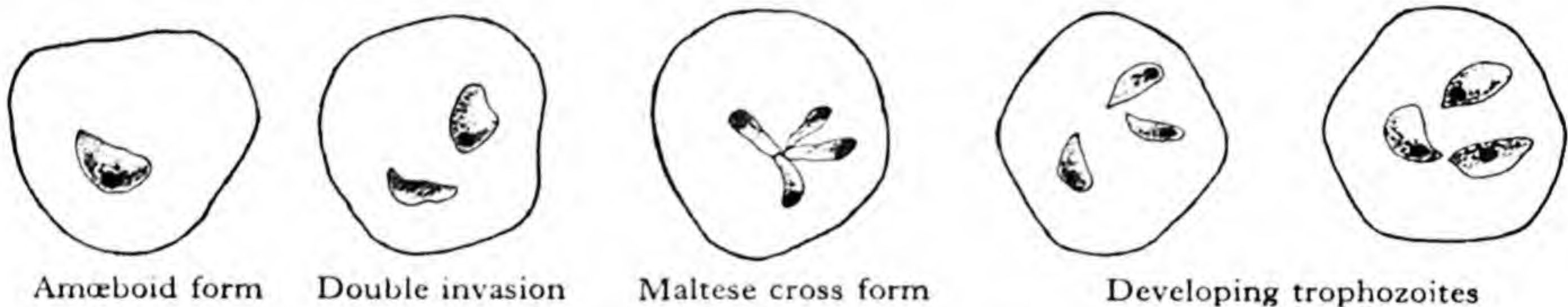


FIG. 28.—*Nuttallia equi*.

Distribution and transmission

The parasite is more widely distributed than *B. caballi*, the range of which it overlaps in Europe and in Russia, in Africa, South America and parts of Asia, including India. It is transmitted by species of *Dermacentor*, *Rhipicephalus* and *Hyalomma*.

Pathogenicity

The organism is regarded as being more pathogenic than *B. caballi* and causes intermittent fever, jaundice and hæmoglobinuria, sometimes in acute form. In Russia, Sassuchin (1933) has described a form of meningitis associated with infection with a parasite which appears to resemble *Nuttallia equi*. Strains of the piroplasm vary markedly in virulence.

Immunity

There appears to be no cross immunity between *B. caballi* and *Nuttallia equi*. Young animals are not so seriously affected

as older ones. In South Africa young animals which have recovered are described as "Salted" and the blood of salted young horses may be used for protective inoculation.

Treatment

Pentamidine has been used with success in France. The acridine compounds have been used successfully in South Africa.

Alexandrov (1958) reports success with trypaflavine, using 150-200 ml. of a 1 per cent. solution.

PIROPLASMOSIS IN SHEEP

As in most other domestic animals a large and a small species of piroplasm are recognised.

BABESIA MOTASI Wenyon, 1926

Morphology

The parasite is a large one, closely resembling *B. bigemina* and measuring from $2.5\ \mu$ to $4.0\ \mu$ by about $2.0\ \mu$. The parasites occur singly or in pairs and, as with *B. bigemina*, the angle at which they meet is usually acute. They are usually pear-shaped. A similar parasite has been described in goats.

Distribution

Southern Europe, Iraq, across Asia to Cochin China, Africa and in other parts of the tropics. Transmission is by species of *Dermacentor*, *Hæmaphysalis* and *Rhipicephalus*.

Symptoms and pathogenicity

The disease may be acute or chronic. In the acute disease, fever, prostration, hæmoglobinuria and profound anæmia are characteristic. Death may result. In the chronic form of the disease there may be no diagnostic characteristics.

Treatment

Trypan blue is effective.

B. OVIS Starcovici, 1893

Morphology

The parasite is very much smaller than *B. motasi*, being about $1.0\ \mu$ to $2.5\ \mu$ in length. Pear-shaped parasites are

comparatively rare and the angle between paired parasites is obtuse as with *B. bovis*. The parasites show a tendency to occupy the margin of the corpuscle. Most are round.

Distribution

Throughout the tropics and in some sub-tropical areas and in Southern Europe. Transmission is by species of *Rhipicephalus*.

Symptoms and pathogenicity

The disease is nearly always less acute than with *B. motasi* but there may be an acute phase accompanied by fever, jaundice and progressive anæmia.

Immunity

There is no cross immunity with *B. motasi*. As with other species of *Babesia* young stock is less susceptible than old.

Vecherkin (1957) describes the immunisation of sheep against *B. ovis* using infected citrated blood taken from sheep at the height of the acute infection. The infection was controlled, if necessary, by the use of trypanflavine or hæmosporidin.

Treatment

Trypan blue has no effect. Acriflavine (Gonacrine) is recommended.

Simic *et al.* (1956) observed rapid recovery in 161 out of 169 sheep infected with *B. ovis*, following a single i/m injection of Berenil at the rate of 3 mg./kg. body weight. The lethal dose was 10 mg./kg.

Other species

Ray and Raghavachari (1941) have reported on a species of *Babesia* found in sheep in India. It is stated to differ from *B. ovis* in being leaf-shaped and being more centrally disposed in the corpuscle.

BABESIA IN SWINE

B. TRAUTMANNI Knuth and du Toit, 1921

Morphology

This is one of the large piroplasms, characterised by its long narrow form. There is usually a large chromatin mass towards the narrow end of the organism and a smaller chromatin granule

at the broad end. Four or more organisms may be present in one red blood corpuscle.

Distribution

The parasite is probably transmitted by species of *Rhipicephalus*. It occurs in Tanganyika Territory, Russia, Italy, Bulgaria and the Belgian Congo.

Shone and Philip (1960) have reported that the African Bush Pig (*Potamochoerus procus maschona*) can harbour *B. trautmanni* for 16 days without showing symptoms.

Symptoms

A chronic benign type of infection with few or no symptoms has been described and a more acute type with icterus, splenomegaly, hæmoglobinuria and a high fever.

Following subcutaneous injection of infected blood the incubation period in the domestic pig is 4-6 days (Shone and Philip, 1960).

Treatment

The disease is reported to respond well to trypan blue.

B. suis

Lanzillo described a piroplasm of pigs in Italy under this name but there does not seem to be any evidence that it differs from *B. trautmanni*.

B. PERRONCITOI Cerruti, 1939

This is one of the small piroplasms. It has been reported from pigs in Sardinia (Cerruti, 1939) and in the French Sudan (Rousselet, 1943). Puccini *et al.* (1958) report successful treatment with "Berenil".

BABESIA IN RODENTS

Babesia rodhaini, Berghe, Vincke, Chardome and Bulcke, 1950

This parasite was discovered in the blood of a wild rodent (*Thamnomys surdaster surdaster*) in the Belgian Congo and was transmitted experimentally to white mice. It has since been maintained by blood passage in white mice in a number of

laboratories. Formerly, experiments on therapeutic substances for the control of piroplasmosis were usually made with *Babesia canis* in puppies. *B. rodhaini* is obviously a far more convenient tool for such laboratory work, but the response of *B. rodhaini* to a particular drug is not always an indication of what will happen with other species of *Babesia*. The parasite produces a very high degree of infection in the erythrocytes (up to 90 per cent. may be infected) and mice usually die on the fourth or fifth day following inoculation.

Unfortunately, an arthropod vector for the parasite has not yet been found.

BABESIA IN CATS

Nuttallia felis of the cat was first described from a wild cat in the Sudan, whilst *B. felis* was described by Carpano in two pumas imported into Egypt from California. A similar species has been described in a domestic cat in India. It is reported to respond to treatment with trypan blue.

CONTROL OF BABESIOSIS

Chemotherapy

(1) *Trypan blue*. In general, trypan blue is extremely effective against the large piroplasms. In particular, it has been widely used for the control of *B. bigemina* and *B. canis*. It has been reported as of use in the treatment of *B. trautmanni* in pigs in the Belgian Congo and for the prevention of clinical outbreaks of *B. caballi* in horses in Russia. The drug is cheap and usually readily available. It suffers, however, from the disadvantage that it must be given intravenously. If, during injection, drug is allowed to escape into the surrounding tissue, a very serious slough will almost certainly result. A pigmentation of the mucous membrane is observed within a few seconds of injection. This colouration may extend to the muscles, resulting in objectionable discolouration in the flesh of animals to be slaughtered in the near future.

The drug is best used as a 1 per cent. solution at the rate of about 5 to 10 ml. for the dog and about 50 to 100 ml. for cattle. It is well tolerated and the dose may be repeated 24 hours later. Usually a single dose is sufficient.

(2) *Quinuronium sulphate and its analogues*

The drugs sold under the proprietary names of "Acapron", "Acaprin", "Pirevan", "Babesan", and "Piroparv" are essentially the same and are Quinol-urea derivatives. They are valuable against the large *Babesias* such as *B. canis* and have been reported as useful against the small piroplasms such as *B. bovis*. Many people prefer to use one of this group against *B. canis* as the drug can be given subcutaneously and there is no danger of a slough as with trypan blue. The drug may cause convulsive reactions in some animals but the effect is usually temporary and passes off in about an hour. Yorke found that a dose of 1 mg. per kg. body weight of "Acapron" is sometimes toxic to puppies. In general, the drug is well tolerated and may be repeated after an interval of 24 hours.

(3) *Phenamidine*

Following Lourie and Yorke (1939), this drug was developed essentially for the treatment of piroplasmosis and has been used with success against both large and small parasites. Its use in the treatment of *B. bigemina* has been reported by Randall and Laws (1947). Workers with other drugs of the group have reported the appearance of relapse strains which have become drug-fast. There is, however, little evidence that phenamidine is more likely to promote drug-fastness than any other agent. In the treatment of *B. bigemina* the drug is well tolerated up to a level of 22.5 mg. per kg. but Randall and Laws (1947) found it toxic at a level of 30 mg. per kg. when used as a 40 per cent. solution.

(4) *Aureomycin*

In 1953 Jansen reported on the parasitocidal effect of repeated doses of aureomycin. Using splenectomised donkeys infected with *B. equi* the drug was reported to be more effective and less toxic than "Gonacrine".

(5) *Acriflavin ("Gonacrine")*

The drug has been reported as of value in the treatment of both types of *Babesia* and in particular is reported to be the drug of choice against *B. equi*. Against *B. bigemina* in cattle, 20 ml.

of a 5 per cent. solution of acriflavin has given good results. This is an excellent drug against *Babesia* but it must be given intravenously.

(6) *Quinine*

Quinine is believed to have some beneficial effect against infection with *Babesia* but in most instances the effect is not marked. It has, however, been used successfully in the form of quinine hydrobromide for the treatment of infection with *Nuttallia equi*. It is given intravenously in doses of 2 to 4 gm. dissolved in 30 ml. of water. Injections must be given very slowly or fatal shock may result.

(7) *Neoarsphenamine*

At a dosage of 0.45 gm. per 40 lb. body weight has been used for the treatment of *B. gibsoni*.

(8) *Ichthargan*

This has been recorded as being valuable in the treatment of *B. bovis* and *B. argentina*, injections being given in doses of 1.0 to 1.5 gm. dissolved in 50 to 75 ml. of distilled water. The dose must be repeated two to three times. Solutions must be prepared as required as they become toxic on standing, and distilled water must be used in preparing the solutions to avoid precipitation of silver chloride. Sergeant *et al.* (1926) found this was the only drug to have any action on *B. major*.

(9) *Todorit*

This preparation is given intravenously in doses of 5 to 10 c.c. It is said to give good results in the treatment of both *B. bovis* and *B. argentina*.

(10) *Diampron*

3:3'-diamidinocarbanilide was examined by Ashley, Berg and Lucas (1960) and by Lucas (1960). The diesthionate is in field use. Therapeutic effect against *B. divergens* (Beveridge *et al.*, 1960), *B. bigemina* (Shone, *et al.*, 1960) and *B. berbera* (Kemron, *et al.*, 1960) has been reported. At the generally recommended dose rate of 5 mg./kg. the drug seems to be well tolerated.

(11) *Berenil*

This drug has been recommended for use against a number of species of *Babesia*. Fussganger (1955) reported that 0.25 mg./kg. was the minimum curative dose against *B. canis* in the dog and 0.1 mg./kg. was the minimum effective dose. Against *B. divergens* Ryley (1957) found it had little effect up to a dose rate of 10 mg./kg. either intramuscularly or subcutaneously and the drug had little effect against *B. canis*. Lucas (1960) also had disappointing results. There was little effect on the course of experimental infection at doses of less than 10/mg./kg. On the other hand Ranali *et al.* (1958) reported that 2/mg./kg. Berenil controlled clinical disease with a mixed infection of *B. bigemina* and *B. argentina*, but even with 3/mg./kg. the blood was not cleared of parasites. In Mexico, Chavarria *et al.* (1958) confirmed the activity of Berenil at a dosage rate of 3/mg./kg. against *B. bigemina* in cattle. Puccini *et al.* found Berenil effective at 3.5/mg./kg. as a 7.0 per cent. solution given intramuscularly against *B. trautmanni* and *B. perroncitoi* in pigs.

The results of such field trials are always difficult to assess and at the moment it seems that Berenil needs further trial under controlled conditions.

Other drugs :—

The chemotherapy of babesiasis is being studied in the U.S.S.R. and a useful review of the literature has been published by Mack (1957). Of a number of drugs mentioned "Hæmosporidin" (N,N'-di-4-dimethyl-aminophenyl) urea methylmethosulphate) was tested against *B. caballi* and *B. equi* in horses, *B. bigemina* in cattle and *B. ovis* in sheep. It appears to be in fairly general use. A related drug, Novoplasmin, is reported as being effective against a number of species of *Babesia*.

CONTROL OF THE TICK

Piroplasmosis can be eliminated only through control of the tick vectors. This is frequently exceedingly difficult, particularly under the special conditions of under-developed countries. In the U.S.A. the disease has been practically eliminated by systematic dipping of all cattle to destroy the principal vector *Boophilus*

(*Margaropus*) *annulatus* together with its Gulf Coast and Florida race *microplus*, yet at one time fourteen States were heavily involved.

In order to appreciate the techniques by which the ticks which transmit piroplasmosis can be attacked it is necessary to understand their life-histories and their relationships with the parasite.

Neitz (1956) has reviewed tick-borne diseases of domestic stock with particular reference to the vectors.

The life-history of the tick

Members of the Family *Ixodidae*—ticks with a hard chitinous shield or scutum—lay their eggs on the ground in sheltered spots, under stones or clods of soil or in crevices. Some species lay up to 20,000 eggs. Development of the egg proceeds at a rate which is influenced by the prevailing temperatures. Cold weather markedly retards hatching. The newly hatched larvæ (seed-ticks) climb onto vegetation and there wait until they are able to attach to a passing host. After engorging with blood the larva moults and passes from a six-legged stage into the eight-legged nymph. After a few days the integument of the nymph hardens. It engorges further and then moults into the adult or *imago* stage. After hardening of the integument the ticks may drop off and copulation occurs on the ground but more often it occurs on the host. The fertilised, engorged female drops off while the male remains on the host for a varying time before it also drops off. On the ground the female lays its eggs in a sheltered spot.

There are several groups of *Ixodidae* a key to which is given in Monnig's *Veterinary Helminthology and Entomology* (1950). From the practical point of view, however, the essential division of the ticks is one based on the life-cycle.

Ticks may be divided into three groups according to whether they spend all the developmental stages on a single host, dropping off only to lay eggs (*one-host ticks*) or whether they spend the larva and nymphal stages on a single host which they leave for the final blood meal as an adult on a second host (*two-host ticks*) or whether they drop off the host after feeding at each of the three developmental stages (*three-host ticks*).

One important difference between the types of ticks is immediately apparent. The one-host ticks which feed only on a single

host throughout their life *must* transmit the *Babesia* through their eggs. Two-or three-host ticks may also transmit infection through the egg, but, in theory at least, they may carry out stage to stage transmission. They can pick up infection from one animal as nymphs, for instance, and transmit it to another as adults. Species of *Boophilus* which transmit *B. bigemina* are all one-host ticks and transmit the infection through the egg. Some, but not all species of *Rhipicephalus* are two-host ticks. Species of *Ixodes*, for example *I. ricinus*, which transmits *B. bovis* in Britain, is a three-host tick. With *I. ricinus* the larva remains on the host for two to six days, the nymph for three to seven days and the adult female for five to fourteen. It is clear that in order to obtain reasonable control of a three-host tick, dipping of cattle must be at frequent intervals. With *Boophilus annulatus*, which remains on the same host for from fifteen to fifty-five days the dipping can be spaced at wider intervals while still ensuring that all ticks are destroyed.

Control measures

Absolute control can be assured only by declaring "clean" areas in which permanent dips or dipping stations are set up and where all animals are compulsorily dipped, initially as frequently as twice a week and later, as the area becomes clear, at fortnightly intervals. The design of the dipping bath must ensure total immersion of the animal, this being usually contrived by a sudden drop into a bath which is about eight feet deep with a total length of about fifty feet and a sloping exit from which the animal emerges into a small pen in which it stands to allow surplus dip to drain back into the tank. It is very important that the dipping tank should be properly constructed. Plans of satisfactory tanks are available from some of the firms which specialise in the sale of dips and these may be modified in the light of local requirements and experience. Spraying of stock with high pressure sprays in specially constructed crushes results in a great economy in dipping material and the most modern types of sprays are reported to be very satisfactory. Under special conditions, *e.g.* in East Africa during the war years when large numbers of cattle were being moved through areas where East Coast Fever was known to be endemic, dressing of cattle in certain predilection sites of the tick (ears and tail) gave a certain amount of protection.

Acaricides

For the dressing of cattle on ears and tail simple, locally obtained materials such as used engine oil and nicotine are reasonably effective. For general use in dips, arsenic trioxide (giving arsenious acid in solution) was, formerly, in general use. Nowadays, arsenical dips are in many areas being replaced or augmented by the newer insecticides. Arsenical dips are cheap, reasonably effective and safe, subject to precautions being taken to avoid the dipping of tired or overheated stock and careful titration of the dip once or twice a week to maintain the correct concentration and to avoid either using a dip which is too weak to be effective or one which is sufficiently strong to produce skin burning. The main objections to using arsenical dips are the extraordinary precautions which must be taken to avoid misuse, and the speed with which, in some areas, resistant strains of tick are developing.

When considering the general problem of tick control it is useful to remember that as the cuticle of the engorging tick stretches it loses its power of actively taking up water and that this, plus the fact that a waxy layer appears, makes the fully engorged tick resistant to acaricides (Theiler, 1959).

Benzene hexachloride

This is best known under the trade name of "Gammexane" which contains the gamma isomer of hexachlorocyclohexane. Benzene hexachloride has proved fully effective at concentrations of about 0.05 per cent. of the gamma isomer particularly against *Boophilus*, both as a dip and as a spray. In combination with arsenic it is in use at concentrations, for dipping, of about 0.01 per cent. to 0.005 per cent.

Toxaphene (chlorinated camphene)

This has been recommended for use particularly against the engorged female ticks. Toxaphene is recommended for use as a spray and as a dip at concentrations of between 0.47 per cent. to 0.7 per cent. Cattle are clear of ticks within nine days of a single dipping and remain clear of infestation with *Boophilus microplus* for twelve days. In East Africa the dip is widely used in the control of ticks in the East Coast Fever areas.

In dips containing 0·5 per cent. toxaphene the particle size of the dispersed phase was observed to increase during the dipping season so that the amount of toxaphene deposited on the coat became greater. This is the suggested explanation of the occasional toxicity of dips containing toxaphene.

D.D.T.

This is the popular name for paradichlorodiphenyltrichloroethane, a powerful but relatively slow-acting insecticide with a very wide application. The substance is virtually insoluble and its maintenance in permanent suspension in the dip presents some technical problems. It is very persistent but is fully effective only against the larval stages of ticks so that it is not very useful against three-host ticks. Until recently it does not appear to have induced the formation of resistant strains of *Boophilus decoloratus*. D.D.T. is commonly used at a concentration of between 0·2 per cent. and 0·5 per cent. of the dip and at this last concentration can kill fully engorged ticks. At 0·25 per cent. D.D.T. can kill unfed and partially fed ticks but it is not effective against half-engorged to fully engorged ticks. D.D.T. is sometimes used in combination with arsenical dips.

Dieldrin

Dieldrin has been used as an acaricide at concentrations of about 0·05 per cent. to 0·1 per cent. but is not particularly effective, as was shown by Wood *et al.* (1960) against *Ixodes ricinus*.

Resistance to dipping

The problem of resistance to dips (particularly with *Boophilus*) is one which is becoming increasingly important throughout the world. All dips which have been in general use seem to be implicated and ticks which prove resistant to one acaricide are likely to prove resistant to others.

Under existing circumstances the best dip to use must be decided under local conditions and if possible dips should be alternated in order to reduce to a minimum the development of resistant strains. Some aspects of resistance to dipping are considered by Fiedler (1952).

The organo-phosphorus compounds

Partly in an effort to control ticks (particularly *Boophilus*) which are resistant to the "chlorinated hydrocarbons" a number of organo-phosphorus compounds have been tested as dips and sprays. Drummond (1961) lists Co-ral (0.25 per cent. and 0.5 per cent.), Delnav (0.15 per cent.), Malathion (0.5 per cent.) and Ronnel (0.75 per cent.) as compounds recommended for use in the U.S.A., where the problem of the residues left by insecticides in animal products is regarded particularly seriously. Legg (1956) showed that Diazinon was particularly effective in killing resistant ticks. Delnav (0.1 per cent.) has been reported as being effective against *Ixodes ricinus* and against *Dermacentor* (0.15 per cent.—Drummond, Moore and Warren, 1959). Asuntol (Bayer, 21/199) has been used at 0.05 per cent. but seems to have an effect against *Rhipicephalus* spp. at as little as 0.01 per cent. to 0.022 per cent. (Behrenz, Federmann and Bolle, 1959). Drummond, Moore and Warren (1959) have reported the use of 0.5 per cent. Co-ral against the cattle winter tick.

CHAPTER XII

OTHER SPOROZOA AND RELATED PARASITES

The next genus *Eggsomella* is very closely related to *Babesia* and *Martinsia* from which it differs in that the forms in the red blood cells divide several times.

The genus *Theileria* includes those unpigmented parasites which are in the reticulo-endothelial system, reproducing by schizogony within cells of the lymphatic system. Certain forms produced in the lymphatic system enter the red cells and appear in the peripheral smear. As in *B. pallens*, contrary to what occurs with other species of *Theileria*, the blood forms do not multiply in the red blood cells. As a rule, if the blood is not infective when examined by smears, it is also infective unless it happens to contain large numbers of parasites.

The genus *Eperythrozoon* is of uncertain relationship. The taxonomist, Dr. B. J. H. Hirst, studies *Eperythrozoon* as a genus of *Babesia*. The *Eperythrozoon* species are found in the leucocytes of many domestic and wild animals and in certain *Eucephalito-*

4. *Leishmania*

Leishmania (L.) KILLIP, 1928 (L.) H. M. C. (1928)

This organism is a parasite of man and animals. A similar organism has been described from an ostrich which had been in contact with humans.

The parasite was first recorded in the Sudan by Ralfour and the parasites were called *Ralfour's granules*. It has since been found in South Africa, India, China and the Balkans and probably occurs in most tropical and sub-tropical countries. It was originally believed that it represented intracellular forms of the food-poison parasite *Trypanosoma amurensis*, which often accompanies the infection. Later, however, it was considered to be a protozoan and was renamed *L. pallidum*.

Morphology

Three forms of the parasite occur in the erythrocytes of fowls—(1) Initial bodies. These are less than $1.0\ \mu$ in diameter and are round or oval. They consist of a chromatin granule with a small halo of cytoplasm. Sometimes no cytoplasm can be detected.

(2) Elements in process of developing. These may be pear-shaped, oval or round. With Leishman they appear as blue-staining cytoplasm containing a chromatin mass stained red and sometimes an accessory chromatin mass. These forms closely resemble *Babesia*.

(3) Large, oval, elliptical or round bodies, $2.0\ \mu$ to $2.5\ \mu$ by $3.0\ \mu$ to $4.0\ \mu$. The chromatin occurs as granules round the

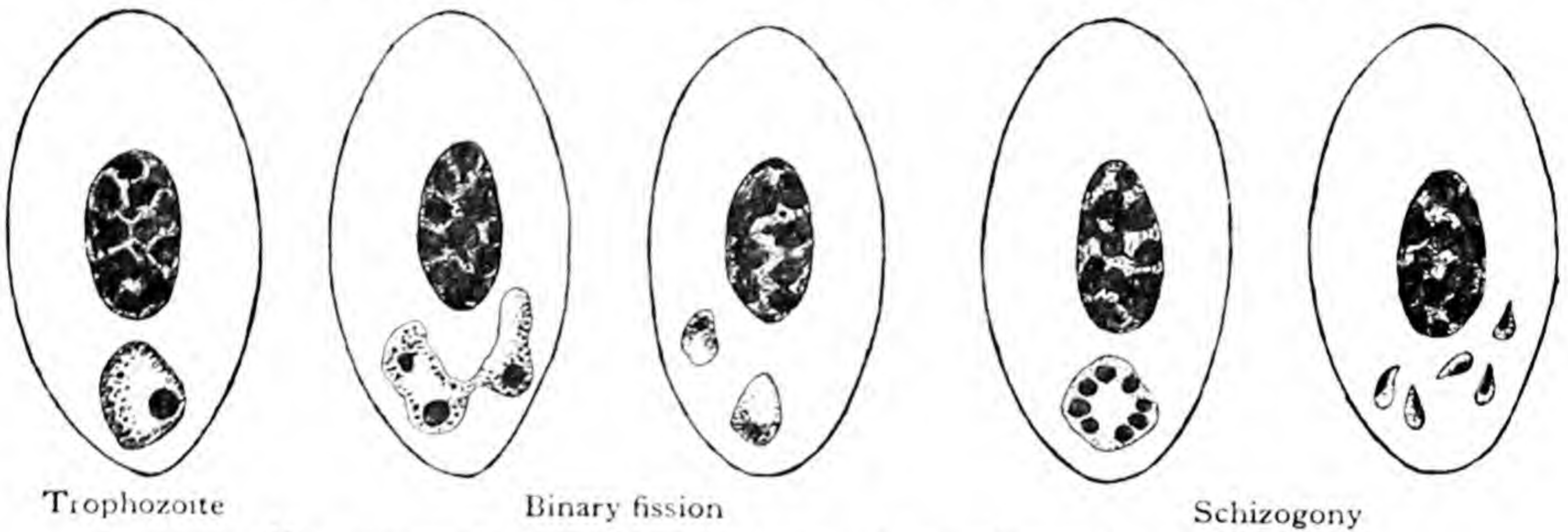


FIG. 29.—*Aegyptianella pullorum* in erythrocytes of fowl.

periphery with a pale accessory nucleus in the centre. These forms are schizonts.

Life-history

There are occasional indications of binary fission but the ordinary means of multiplication appears to be by schizogony, the schizonts breaking up into four to sixteen merozoites. The cytoplasm of the red cell breaks down, setting free the merozoites. Sometimes large forms develop giving no evidence of division and possibly representing gametocytes.

Transmission is through the tick *Argas persicus* but the development in the tick has not been followed.

Pathogenicity

The disease is very often associated with fowl spirochætosis. When apparently uncomplicated in this way it can be divided into

acute, subacute and chronic forms largely depending on the susceptibility of the host. Fowls native to the area rarely have the acute disease but freshly introduced stock may die in a few days with diarrhœa, anorexia, a high temperature and paralysis. Post-mortem examination shows anæmia, splenic tumours and punctiform hæmorrhage in the serosa.

Treatment

According to Sargent (1935) ichthargan given intravenously is a specific against the condition.

THEILERIA

Species and classification

The species of Theileria in livestock cannot be distinguished one from the other on morphological grounds but differ in pathogenicity, in the fact that infection with one does not confer immunity to another and in their vectors. Morphological differences between species have sometimes been reported but are usually quantitative differences, *i.e.* where polymorphism exists the proportion of the different forms may apparently vary between different species. Such differences are difficult to apply in practice.

The species in cattle have been studied to the greatest extent mainly because *T. parva* is of great importance in East Africa. They have been divided into three groups :—

- (a) The *mutans* group, with *T. mutans* as the only species.
- (b) The *annulata* group, *T. dispar* being often regarded as a synonym for *T. annulata*. (The systematic position of *T. sergenti* and *T. turkestanica* is obscure.)
- (c) The *parva* group to which belongs *T. parva*, the apparently closely related *T. lawrencei* and the possibly synonymous *T. bovis*.

Neitz (1956, 1957 and 1959) has extensively reviewed the group and has produced evidence for the separation of *T. parva* from the rest of the species and the creation of the Family *Gonderidæ* (Neitz and Jansen, 1956) with the genus *Gonderia* (du Toit, 1918) to include the species *annulata* and *mutans* and the new species *lawrencei* and *bovis*. It is proposed in this book to retain all species in the genus *Theileria*.

The forms found in red blood corpuscles of livestock comprise rings, ovals, rods or comma-shaped bodies about $0.5\ \mu$ to $1.0\ \mu$ broad by $1.5\ \mu$ to $2.0\ \mu$ long. Stained by Giemsa they show a red chromatin granule at one end and a blue cytoplasm. Infection of a corpuscle is commonly multiple and except with *T. parva* there is evidence that multiplication of these forms occurs. The actively multiplying forms of the parasite occur in the endothelial cells and the lymphocytes, particularly in the lymphatic glands and spleen. The dividing forms, which are regarded as schizonts, are known as *Koch's Blue Bodies* and occur as circular or irregularly shaped structures varying in diameter from $2.0\ \mu$ to $12.0\ \mu$ or more, either contained in the cytoplasm of the leucocytes or free in the gland or spleen juice. Dried smears stained with Romanowsky stains contain blue masses of cytoplasm with a varying number of red chromatin dots. The size of the granules varies. The Koch's Blue Bodies can be seen to break up into uninucleated merozoites about $1.0\ \mu$ broad by $2.0\ \mu$ long, or into the rather smaller forms which enter the red blood corpuscles. The schizonts may escape into the blood and can sometimes be detected in blood smears.

Life-history

This has been most fully studied in the case of *Theileria parva*. The stage infective for cattle is a small uninucleate sporozoite occurring in the saliva of infected ticks such as *Rhipicephalus appendiculatus*. Sporozoites are injected into the ox when the tick sucks blood, and travel by way of the lymphatic system to the lymphatic glands and other centres of lymphatic tissue. There they enter the lymphocytes and occasionally the endothelial cells, increasing in size and forming the schizonts (Koch's Blue Bodies) previously described. These, characteristically, occur in the cytoplasm of the leucocyte, but on disruption of the host-cell are found free in the lymphatic fluid. The schizonts occur in two forms, those with large chromatin granules, known as *agamonts*, (macroschizonts) which break up into merozoites which repeat the schizogony process and those with small chromatin granules, known as *gamonts* (microschizonts) (Barnett, *et al.*, 1961) which break up into the minute uninucleate forms which invade the red blood corpuscles. These latter forms do not undergo further

development until ingested by the tick and are believed to be gametocytes.

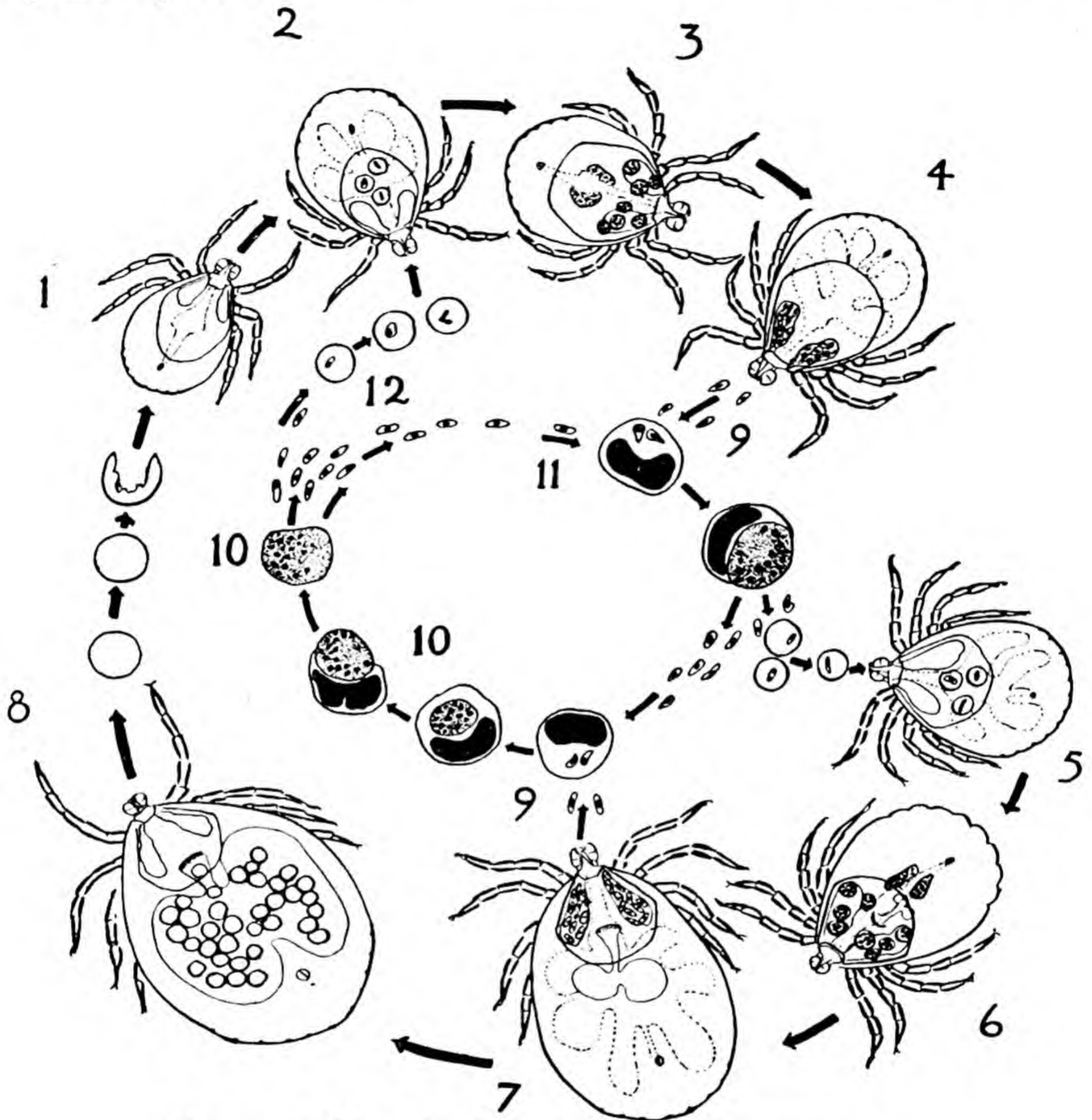


FIG. 30.—Life-cycle of *Theileria parva* (diagrammatic).

1. Clean larva of *R. appendiculatus*.
2. Larva ingests infected blood of ox.
3. Larva moults to nymph. Parasites migrate to salivary glands.
4. Infected nymph transmits infection to clean host.
5. Clean nymph ingests blood of infected host.
6. Infected nymph moults to adult. Parasites migrate to S. glands.
7. Infected adult transmits infection to clean host.
8. Infected female lays eggs which hatch to clean larvæ.
9. Parasites from tick penetrate lymphocytes.
10. Schizonts form in lymphocytes as agamonts or gamonts.
11. Merozoites from agamonts invade other lymphocytes.
12. Gametes from gamonts invade erythrocytes.

When ingested by the tick, in the larval or nymphal stages, the parasites are freed from the corpuscles and congregate in masses in the intestine of the tick. A process of syngamy has

been described by Gonder (1911 *a* and *b*) but no such process was noted by later investigators (Cowdry and Ham, 1932). The next form to appear is a large organism with an irregular outline and no distinct nucleus which has nevertheless been described as a zygote. At about the time of formation of the zygote the tick drops off the host. The nucleus of the zygote reappears and divides to form a multinucleate organism, the *ookinete*, which is motile, penetrates the intestine of the tick and comes to rest in the body cavity. When the tick completes its moult the ookinete migrates to the salivary gland where it commences to bud off multinucleate masses, the sporoblasts. When the tick attaches itself to the new host and commences to suck blood, the sporoblasts break up into uninucleate sporozoites which enter the salivary duct and are injected into the new host. It appears that the act of feeding actually induces the development of the sporozoites from the sporoblasts in the salivary glands. They pass into the blood stream on the third to fifth day of attachment, degenerating if the animal is not susceptible. It appears that infection cannot be produced until the tick has been attached for three days.

With *T. parva* developing in *R. appendiculatus* infection must be taken up by the larva or nymph and transmitted to the nymph or adult, parasites not surviving more than one moult, so that an infected tick loses its infectivity if it attaches to a non-susceptible animal.

Reichenow (1938), as the result of investigations in Tanganyika, claims that there is no development of *T. parva* in the gut of the tick. He asserts that the ingested parasites pass directly to the salivary glands where they lie dormant whilst the tick falls off the host and moults. When the next-stage tick commences to feed, multiplication occurs by binary fission in the cells of the salivary gland which eventually rupture, the parasites being released into the salivary ducts. This process takes three days. He ascribes the variation in size of the chromatin granules of the Blue Bodies to changes in the nuclei occurring at various stages of development and points out that the parasites liberated from the lymphocytes are all alike. He suggests that parasites entering the blood stream find only a few lymphocytes to invade and therefore invade the red blood corpuscles in which they are unable to multiply; they therefore lie dormant till ingested by a suitable tick.

T. PARVA Theiler, 1904

This parasite causes the disease occurring in East, Central and South Africa and known as East Coast Fever, an extremely serious disease with a high mortality among susceptible stock. The parasite is pathogenic only in cattle, although some species of Hartebeeste and Gazelle have been reported to harbour infection. *T. parva* differs markedly, in several respects, from the other species of the genus. Thus, inoculation of blood from an infected animal will not as a rule set up infection in a susceptible

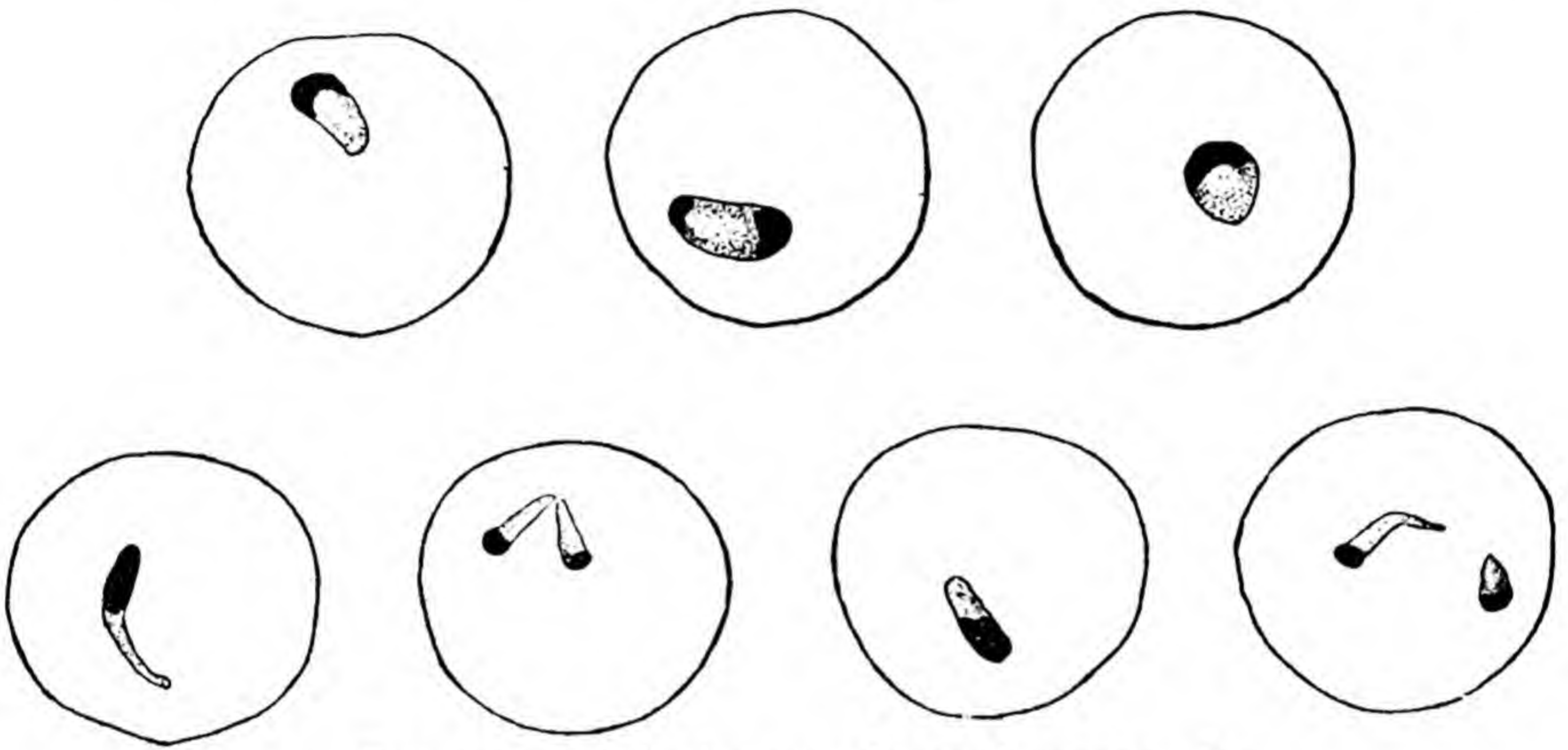


FIG. 31.—*Theileria parva* in erythrocytes of ox.

animal although Theiler and du Toit (1929) succeeded in transmitting the disease irregularly by blood injection. Recovery from infection is characterised by the development of a solid immunity which is nearly always sterile. Consequently, ticks fed on a recovered animal do not become infective to other cattle. In all these characters *T. parva* differs markedly from, for example, *T. mutans* in which resistance is associated with premunity and in which transmission is more readily effected by blood passage. On the other hand, *T. parva* is readily transmitted intravenously or intraperitoneally by spleen and lymph gland suspensions. It should be noted that Koch's Bodies very often appear in the peripheral circulation. To explain the solid immunity to *T. parva* Richardson (1930) and Neitz (1943) and (1946) have suggested the possibility of a second agent, a "virus", being involved.

Symptoms and pathology

About ten to twenty days following exposure to infection the disease commences with fever which continues until death or recovery takes place. All the superficial lymph glands become hypertrophied but during the early stages of the disease the animal retains condition and continues to feed. About a week after the febrile symptoms are apparent the animal may die showing marked leucopenia, the occurrence of kidney infarcts and œdema, particularly of the lungs. This œdema is probably the immediate cause of death.

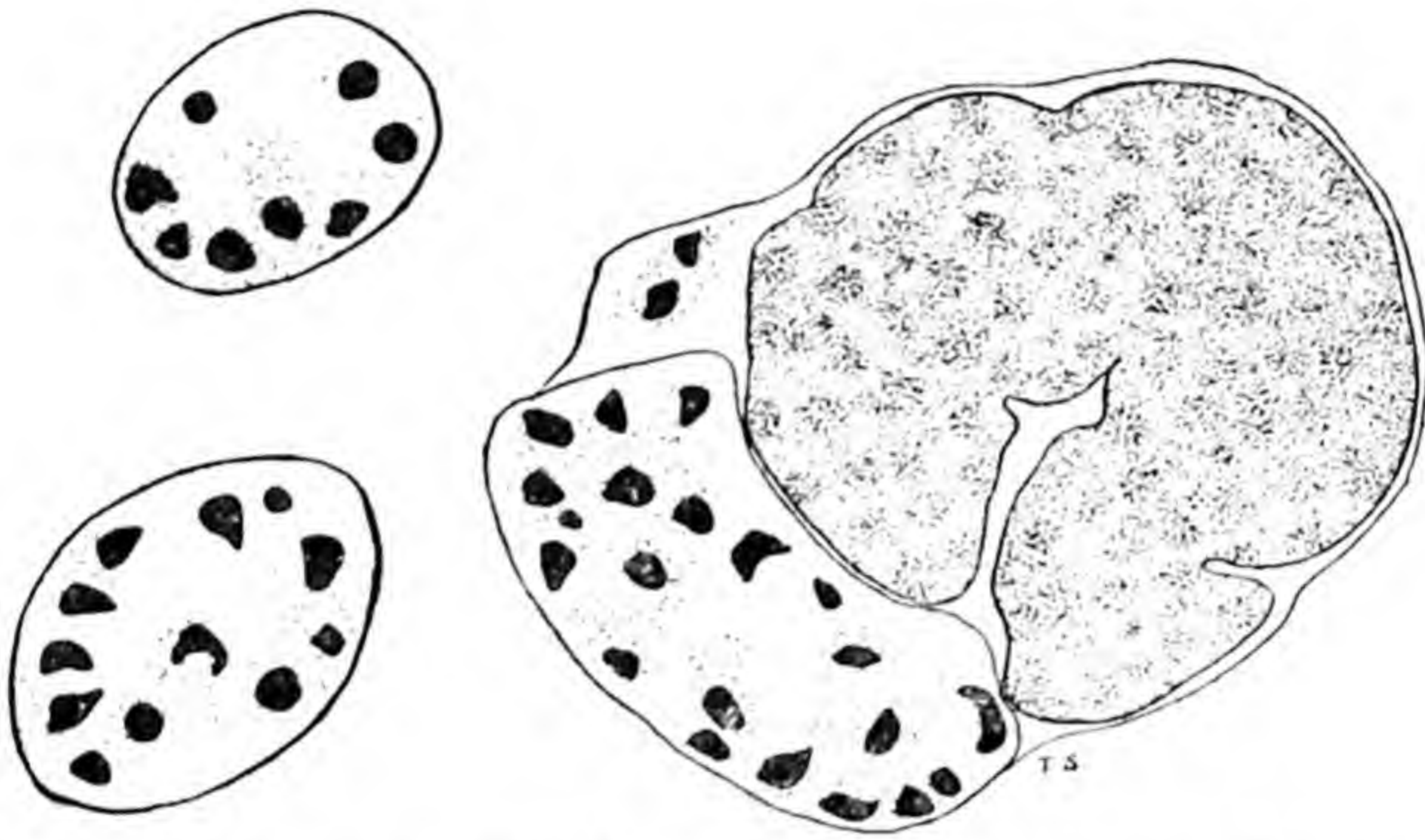


FIG. 32.—“ Blue bodies ” of *T. parva* in cytoplasm of monocyte and free in lymph.

Hæmorrhagic patches may occur in the mucosæ and on the serous membranes and there may be a general swelling of the subcutaneous tissues. It is possible that death results from the elaboration of toxic substances which selectively affect the endothelial cells of the blood vessels and the renal epithelium. Mortality may be as high as 80 to 90 per cent. of the cattle population. Studies on the pathogenesis of East Coast Fever have been reported by de Koch (1957).

In East and Central Africa the disease called “Turning sickness” (Mettam and Carmichael, 1936) in which the animals make circling movements with abduction of the hind limbs has been ascribed to infection with *Theileria parva*. Only a moderate degree of fever occurs. There is incoordination of movement and the animals blunder into fixed objects. Death occurs in up

to fourteen days after the onset of symptoms. There is an increase of cerebro-spinal fluid, the meningeal vessels show stasis and large areas of the brain cortex show extensive blood extravasation. Pin-point hæmorrhages are commonly observed in the white matter and hæmorrhages occur into the roof and wall of the lateral ventricles. The choroid plexus is congested. Necrotic areas occur in the brain tissue and there are bodies resembling the schizonts of *Theileria*. The condition has only been encountered in areas where East Coast Fever is endemic but the epizootiology is not entirely clear. Bovine cerebral theileriosis has been reported by Flanagan and le Roux (1957).

Diagnosis

Under field conditions the detection of *T. parva* in the erythrocytes is not always easy and diagnosis is ordinarily through the demonstration of Koch's Blue Bodies in the fluid obtained from a superficial lymph gland with a long stout needle. On post-mortem examination the schizonts can similarly be identified in the spleen. Specific diagnosis is on the basis of the clinical picture, the post-mortem appearance and the epidemiology. Differentiation of the blood forms on morphological grounds is impossible.

Epidemiology

The incidence of the disease is related to the incidence of the principal vector *Rhipicephalus appendiculatus*. In areas where the tick is numerous the local cattle population is resistant to further infection as the result of contracting the disease when young. It has usually been believed that in endemic areas a high proportion of the calf crop is lost annually as the result of East Coast Fever but Barnett and Bailey (1956-1957) found that in one endemic area in Kenya the mortality was not formidable. Out of a total calf mortality (up to one year) of 13·7 per cent. only 3·7 per cent. died of E.C.F. The immunity which results from infection with *T. parva*, unlike that resulting from some other protozoal infections is solid and does not depend on a condition of premunity. Heifers reared in endemic areas in East Africa have always commanded a high price owing to their strong resistance to E.C.F., when moved to other areas. Barnett and Bailey (1956-1957) studied

the duration of immunity to *T. parva* and found that three years after recovery half the animals were still completely immune while the others had a substantial degree of immunity. Under natural conditions (in addition) the immunity is constantly being reinforced.

Treatment and Control

Formerly no treatment seemed to have an effect on this species. Neitz (1953), however, reported that there was some effect with aureomycin. There was a marked decrease in the schizonts in the lymph nodes although the erythrocytic stages were unaffected. Apart from a mild febrile reaction and swelling of the superficial lymph nodes no experimentally infected and treated animals showed evidence of disease. *Pamaquin* is supposed to cause degeneration of the blood forms of the parasite although it does not appear to affect the clinical picture. *R. appendiculatus* larvæ may fail to become infected after feeding on treated cattle. Control is by control of the tick vector which, as indicated, is usually *R. appendiculatus*. This tick has certain predilection sites on cattle, particularly the ear, and dressing of the ear with insecticides often causes a marked reduction in mortality particularly with susceptible cattle moving through endemic areas on stock routes.

The ticks which transmit *T. parva* are two-or three-host ticks, the infection being acquired by one stage of the tick and transmitted by the next. If the infective tick attaches to a species of animal which is not susceptible to infection with *T. parva* then the tick loses its infectivity. It is believed that the infection is not passed through the egg, so that tick control measures are likely to be effective. With three-host ticks, however, the dipping to which reference is made in the chapter about *Babesia* needs to be carried out at three-day intervals, which is not always practicable in range cattle. In settled areas, where effective police measures can be implemented, ticks can be controlled by regular dipping and the disease brought under control. In Southern Rhodesia, a method of control has been developed which aims at removing the infected animals and preventing infected ticks reaching new hosts. All animals in the infected herd are examined daily and any showing fever are slaughtered, the herd being

moved to a clean area three times at intervals of sixteen days. Because it requires more than sixteen days for larvæ or nymphs to engorge, moult and re-attach themselves, all infected ticks will be left behind at each move and because the longest incubation period is twenty-five days, no new cases capable of infecting ticks should occur after the second move. Ticks will still be present, but none of them should be infected and no source from which they can obtain infection should be available. As, however, infected larvæ and nymphs will have dropped off in the areas used for the first two moves these areas should not be restocked with cattle for eighteen months. The majority of larvæ and nymphs of *Rhipicephalus appendiculatus* are, however, believed to survive unfed for about six months only, and if they feed on non-susceptible hosts such as sheep or game, they clean themselves of infection. Adult ticks may live for as long as eighteen months after having been infected as nymphs. The artificial passage of the disease by the intravenous injection of spleen pulp, in an effort to stimulate immunity, has been practised for many years but had the disadvantages inseparable from a disease for which no adequate treatment was known. Barnett and Bailey (1956-1957) have, however, described a process of immunisation whereby the infection was suppressed to a mild febrile reaction with very little clinical evidence of disease by the use of aureomycin. The method was not suitable for calves less than three months of age.

THEILERIA ANNULATA Dschunkowsky and Luhs, 1904

Morphology

The blood form is indistinguishable from *T. parva* but the parasites are more commonly found in minute ring forms. Macroschizonts and microschizonts can be demonstrated. Schizonts are fairly numerous in the circulation and the parasite, unlike *T. parva* is, at certain times, at least, readily transmitted by blood passage.

Distribution

T. annulata occurs widely throughout tropical and subtropical Africa, Asia and Southern Europe where it causes Mediterranean

Coast Fever. There is a report from Argentina. Transmission is by species of *Hyalomma*. A number of strains of varying virulence has been described. Similar parasites have been identified in zebu cattle, water buffalo and the American bison. *T. dispar* is probably a synonym. Congenital infection has been described by Tsur and Krieger (1960).

Symptoms and pathogenicity

Schizonts appear in the parotid lymph glands 7-18 days after the ears of susceptible cattle have been infested with ticks containing *T. annulata*. A thermal reaction after 2-3 days indicates that parasites have invaded the blood.

Erythrocytic parasites appear 3-4 days after the initial rise in temperature.

The disease occurs in acute, subacute and chronic forms. Severe disease is characterised by fever, depression, lachrymation and tumefaction of the eyelids, rapid emaciation and sometimes hæmaturia (Sergeant *et al.*, 1926). Barboni (1942) reported that 2 per cent. of the infections with this parasite in the Tiber Valley were associated with necrotic hæmorrhagic infarcts of the brain, most numerous in the white matter. This complication was usually fatal. Usually calves suffer a symptomless infection but pathogenicity has been reported in calves in Iraq. The disease is common in Turkey. A high temperature of 40° C. to 42° C. is observed, with an increased pulse rate. There is diarrhœa and sometimes nervous symptoms and death may occur within a few days. In more chronic cases there may be jaundice. On post-mortem examination there are intestinal hæmorrhages with ulceration. The lesions occurring throughout the organs are suggestive of a toxin.

Tsur-Tchernomoretz, Davidson and Weissenberg (1960) report some cases of bovine theileriasis with cutaneous lesions. Large numbers of Koch's Blue Bodies were found in the dermis.

Immunity

Infection with *T. annulata* leads to the development of a condition of premunity with the possibility of relapse following such stress factors as intercurrent disease. Infection is readily transmitted by blood passage.

Treatment and control

Intravenous inoculation of acriflavin (Gonacrine) at the rate of 20 ml. of a 5 per cent. solution has been recommended and among other drugs reported to have some effect are Lomidine (dimethane sulphonate of 4-4' diamidino-diphenoxypentane) and diethyl-amino-methyl butyl-amino-chloroquinoline. In Yugoslavia, an intravenous injection of formalin, 5 ml., with saline or glucose saline, 45 ml., repeated in 24 hours, has been reported to assist recovery. In Israel calves are vaccinated against the local virulent strain of *T. annulata* using a mild strain from Algeria. Infected citrated blood is best used within three days.

As suggested by Daubney and Said (1951) the species of *Hyalomma* which act as vectors hibernate in buildings and under such circumstances may be more susceptible to control than are, for example, species of *Rhipicephalus*.

THEILERIA MUTANS Theiler, 1906

Morphology

The parasite is indistinguishable from the other species of *Theileria* but schizonts in the lymph glands are comparatively rare. It is not transmitted by *Hyalomma mauritanicum* as is *T. annulata*.

Distribution

Distribution is probably world-wide. Needle passage of blood from cattle infected with *Babesia bovis* by Hignett (1953) showed the presence in British cattle of a parasite which he considered indistinguishable from *T. mutans*. The parasite was only about 1 μ in length and there were often two to four parasites in a single blood cell. The schizogonous stage was not identified.

The principal vector in South Africa is *Rh. appendiculatus* but the parasite is believed to be transmitted by a variety of ticks.

Symptoms and pathology

T. mutans is usually regarded as non-pathogenic but there are occasional reports of severe disease caused by a *Theileria* in areas where the presence of the more pathogenic species has not been

reported. With the severe disease, schizonts are numerous. According to Wenyon (1926) these schizonts are larger than those of *T. parva*. *Tsaneen* disease in South Africa is believed to be caused by a virulent strain of *T. mutans*.

Transmission and immunity

Although ordinarily tick-borne, *T. mutans*, like *T. annulata*, is readily transmitted by blood inoculation. Infection is followed by resistance to further attack, with the parasites harboured in a state of premunity.

Treatment and control

None are ordinarily necessary. Tick control should be recommended as with *T. parva* if a pathogenic strain of *T. mutans* exists.

THEILERIA LAWRENCEI Neitz, 1955

This parasite causes a fatal disease in cattle as reported by Neitz *et al.* (1955) in Zululand and by Barnett and Brocklesby (1959) in Kenya. Owing to the scarcity of grazing, cattle were moved off certain European-owned farms into an adjoining game reserve (Neitz, 1955). During the following three weeks about half the animals died, the rate of mortality of those affected being about 90 per cent. Koch's Blue Bodies, relatively smaller than those of *T. parva*, were found in preparations from the affected herd. As no cattle had been kept in the area it seemed that infection must have been derived from the wild game. Ticks collected from the affected pasture by dragging, set up the disease in experimental animals but the disease could not be set up by experimental tick-transmission from infected to healthy cattle and when the remaining cattle were returned to their home farms the mortality ceased. It is thought that this "Corridor disease" is probably the same as the disease described in Southern Rhodesia as "Buffalo disease". The parasite appears to persist in the peripheral circulation of the buffalo (*Syncerus caffer*) for at least four months and nymphal *Rhipicephalus appendiculatus* fed on this animal transmitted the infection in the next stage. *T. lawrencei* multiplies by schizogony in the lymphocytes and by

binary fission in the erythrocytes giving rise to four-daughter individuals (Neitz, 1956). According to Barnett and Bailey (1956-1957) eye lesions are more noticeable with *T. lawrencei* infection. The white cell count usually falls lower with *T. parva*. There is at least some degree of cross immunity between the two species.

TABLE XIV. SPECIES OF THEILERIA

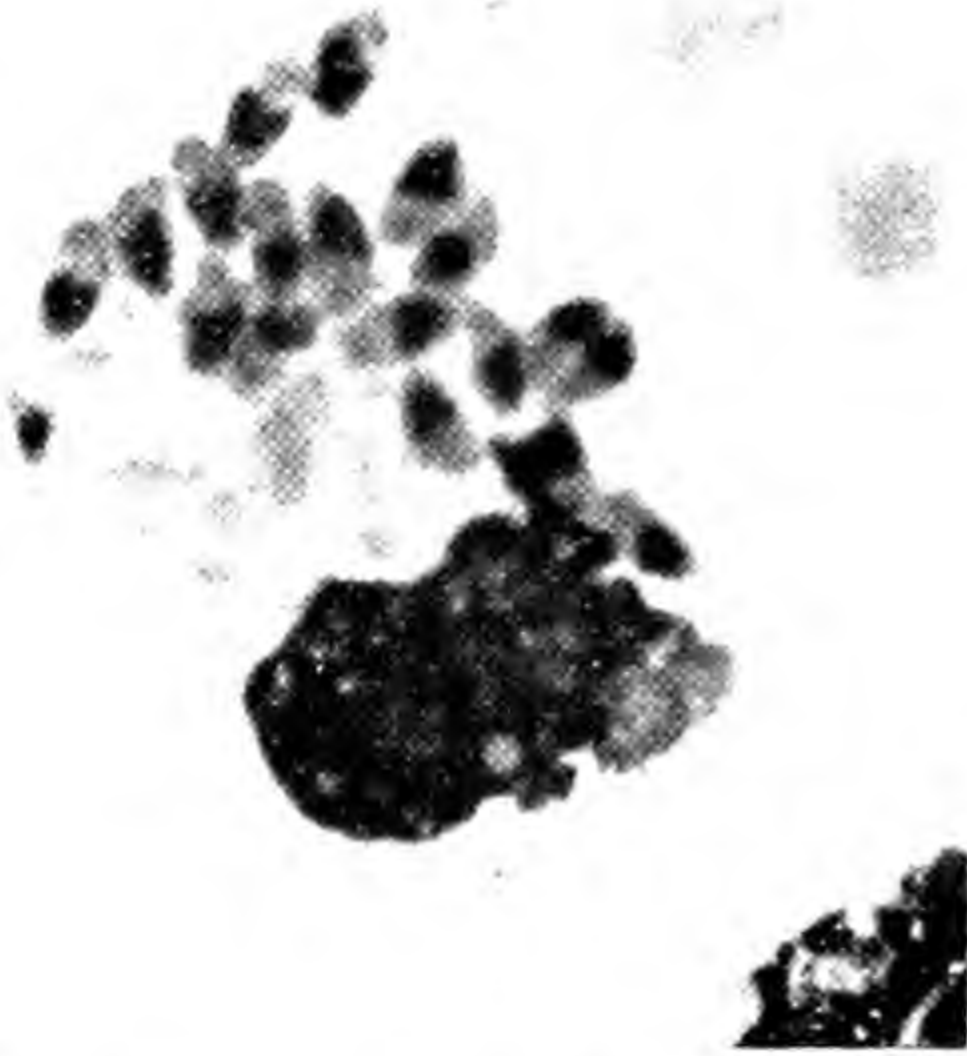
Host	Species	Synonyms	Principal vector	Other vectors
Ox	<i>T. parva</i> Theiler, 1904	...	<i>Rhipicephalus appendiculatus</i>	Several species of <i>Rhipicephalus</i> . Also <i>Hyalomma</i> sp. (experimentally)
	<i>T. annulata</i> Dschunkowsky and Luhs, 1904	<i>T. dispar</i> Sergent <i>et al.</i> , 1924	<i>Hyalomma mauritanicum</i> (Algiers)	Several species of <i>Hyalomma</i>
	<i>T. mutans</i> Theiler, 1906	<i>T. sergenti</i> Yakimoff and Soudatschenkoff, 1931	<i>R. appendiculatus</i>	Several species of <i>Rhipicephalus</i> . Also <i>Boophilus</i> sp. (?)
	<i>T. lawrencei</i> Neitz, 1955	<i>T. bovis</i> (?) G. bovis Neitz, 1957	<i>R. appendiculatus</i>	...
Sheep and Goats	<i>T. ovis</i> Rodhain, 1916	<i>T. recondita</i> Lestoquard, 1929	<i>Rhipicephalus bursa</i>	<i>R. evertsi</i> and <i>Ornithodoros lahorensis</i>
	<i>T. hirci</i> Dschunkowsky and Urodshevich, 1914	<i>T. ovis</i> du Toit, 1918	<i>R. bursa</i>	<i>Ornithodoros lahorensis</i>

GONDERIA (THEILERIA) BOVIS Neitz, 1957

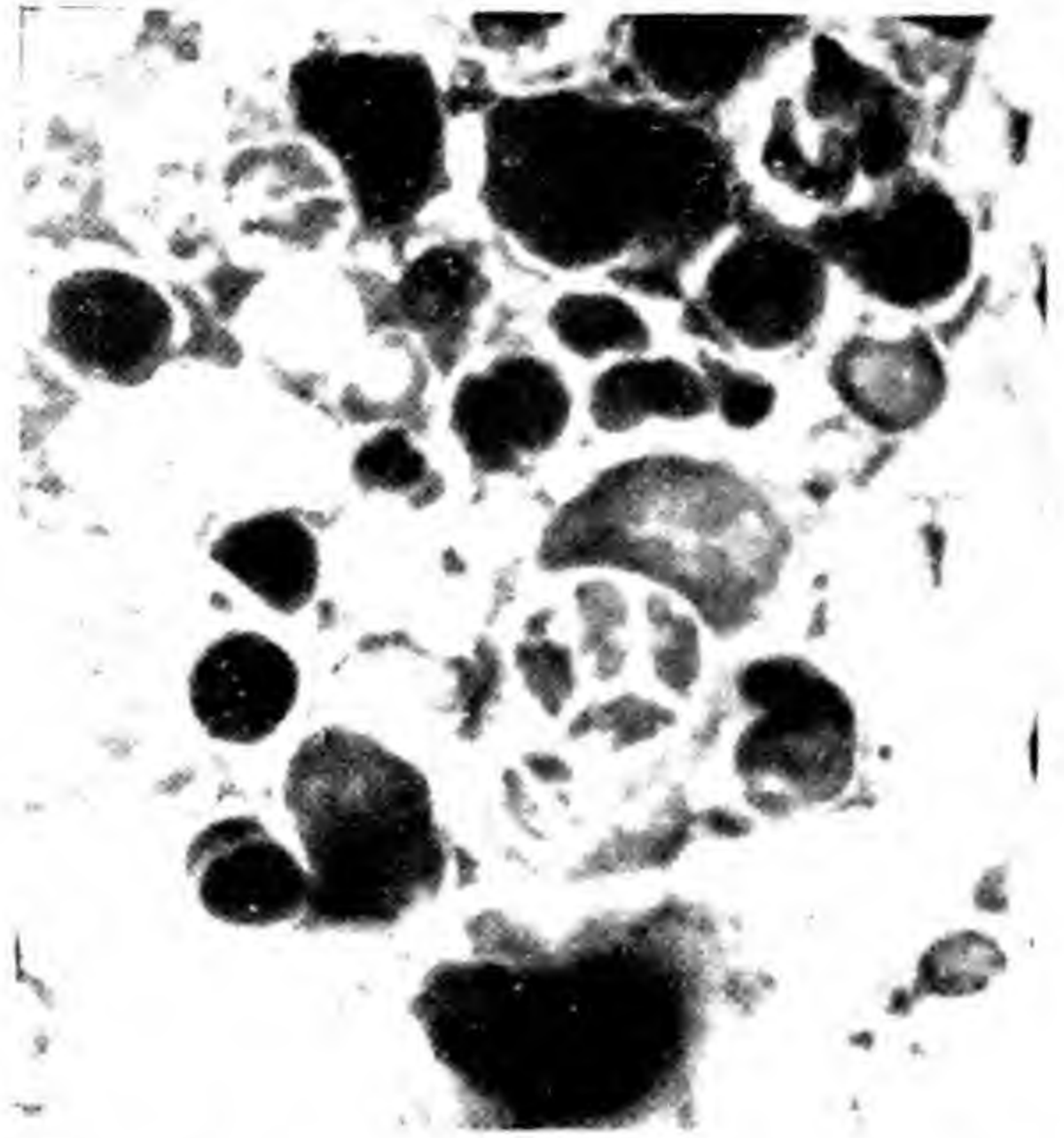
This species, which is stated to be responsible for Rhodesian Malignant Gonderiosis is not readily distinguishable from *T. lawrencei*. The disease is characterised by pyrexia, anorexia, malaise, a variable degree of lymphadenitis, general weakness, prostration and dyspnoea before death (Neitz, 1957).

THEILERIA IN ANIMALS OTHER THAN CATTLE

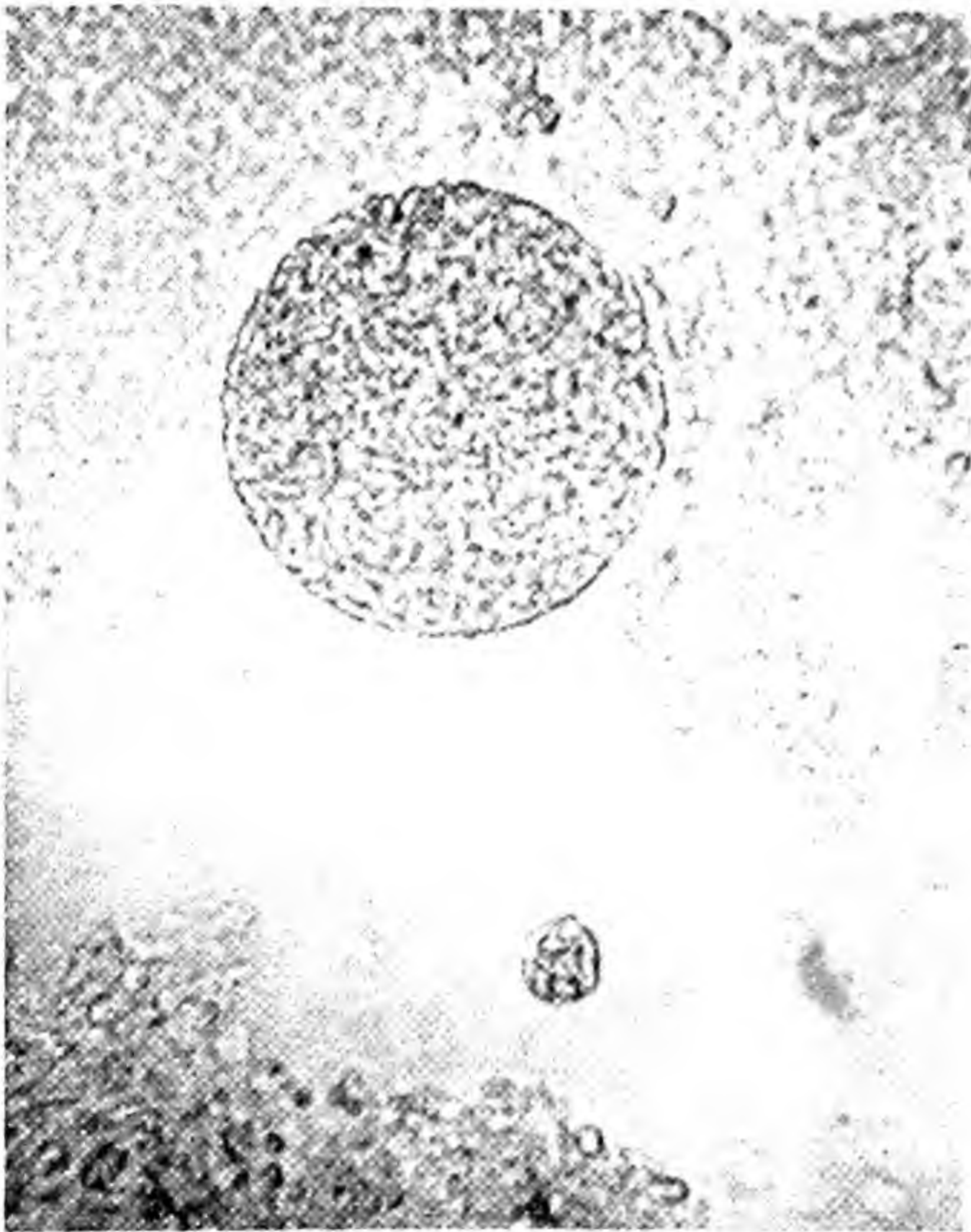
Two species of *Theileria* have been described in sheep and goats. *T. ovis*, reported from America, Asia and Europe, resembles *T. mutans* in that pathogenicity is very low and schizont formation is not normally detected. *T. hirci*, found in N. and E.



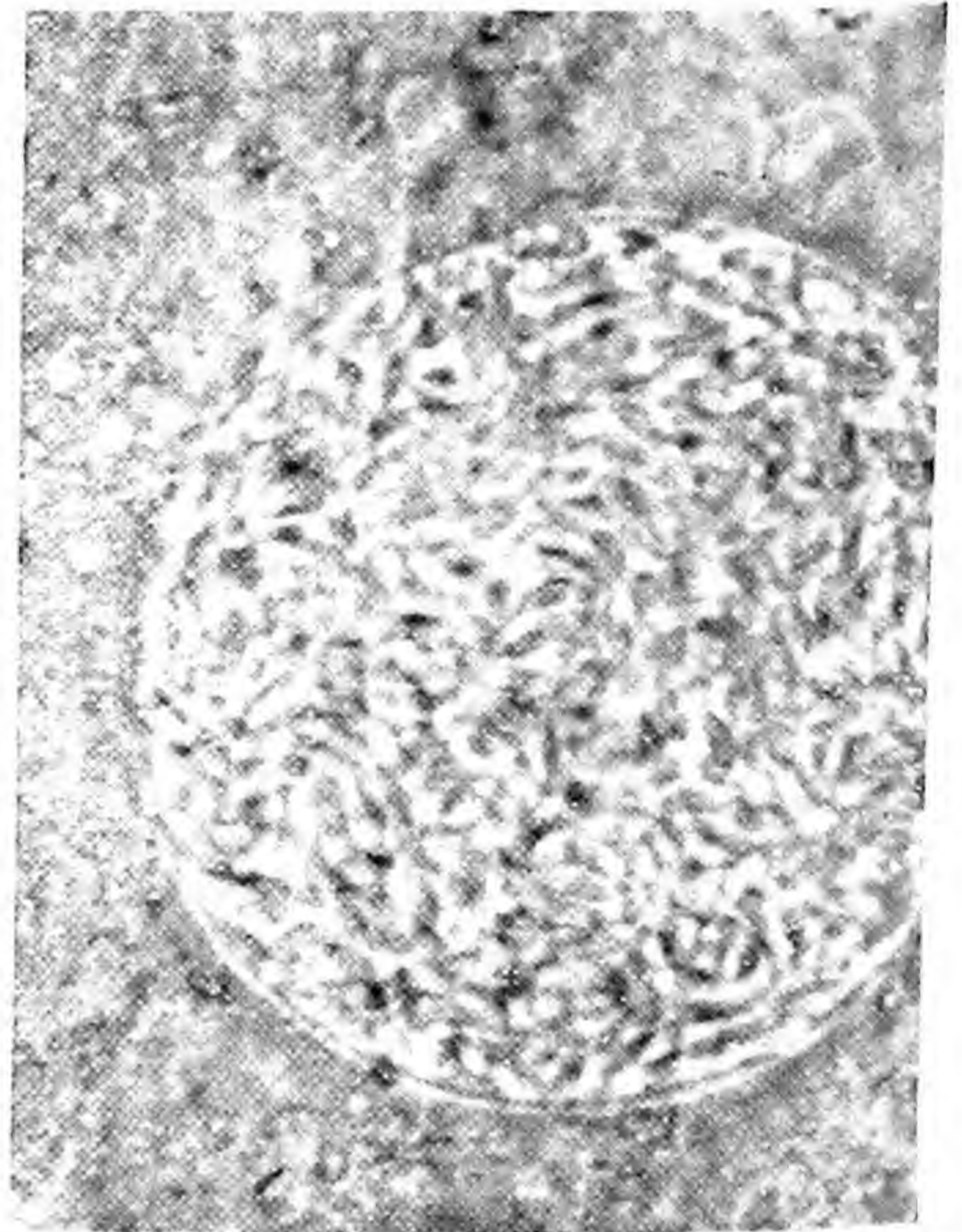
Proliferative forms of *Toxoplasma*
liberated from host cell



Pair of toxoplasms and group of
four toxoplasms inside vacuoles
in monocyte



Old and young cysts of *Toxoplasma*



Large cyst squashed to show contained
toxoplasms

Africa, Iraq, Turkey, the U.S.S.R. and Greece is of a comparable pathogenicity to *T. annulata*. Highly fatal disease may occur, characterised by nasal discharge, jaundice, subcutaneous, submucous and subserous petechial hæmorrhages, enlargement of the spleen and lymphatic glands and enlargement of the kidneys which may show infarcts similar to those of East Coast Fever (Baumann, 1939).

Infections have been reported also from various African ruminants. Neitz (1959) has listed one species of *Theileria* and five of *Gonderia* in domestic animals and 10 named and 36 unnamed species of *Theileria*, one species of *Gonderia*, and two of the related *Cytauxzoon* in wild animals. It has been suggested that the African buffalo (*Syncercus caffer*) may contract a true East Coast Fever infection, probably in a mild form and that it is susceptible also to *T. mutans* and *T. lawrencei* (Neitz, 1957). The Indian Water-buffalo also is reported to be susceptible and the death of an American buffalo from an infection with *Theileria* has been reported from Cairo. In view of the difficulty of species differentiation it is clearly very difficult to be sure just what these parasites were.

In general there are no very satisfactory drugs for use against these species of *Theileria*. Mack (1957) has published a useful review of the chemotherapy of both piroplasmosis and theileriasis of farm animals in the U.S.S.R.

TOXOPLASMA

Toxoplasma was first observed as an intracellular parasite of the spleen and other organs of the African rodent the gondi (*Ctenodactylus gondi*). Since that time increasing interest has been aroused owing to its identification as a pathogen in a large number of hosts, including man. There is at present a very large literature on the subject. There is a good general account by Jacobs (1953) and by Hoare (1956).

The name *Atoxoplasma* has been proposed by Garnham (1950) for toxoplasma-like organisms which are host-specific in birds.

Morphology

In shape *Toxoplasma* resembles the merozoite of a coccidium. It is crescentic, with one end attenuated and the other more

rounded. Its dimensions vary between $2\ \mu$ to $4\ \mu$ in width and $4\ \mu$ to $6\ \mu$ in length. Its nucleus is situated nearer the rounded end. There is no kinetoplast and no centrosome but a chromatic granule is sometimes present. The nucleus has been variously described as composed of granules of chromatin in the form of a loose network or as vesicles with chromatin particles arranged peripherally. No pigment is present at any stage. The organism stains well with Wright's and with Giemsa stains (cytoplasm pale blue, nucleus dark) and may be differentiated from *Encephalitozoon* on the basis of the staining reaction. For example, in tissue sections, *Encephalitozoon*, by contrast with *Toxoplasma*, stains uniformly pale pink or blue with hæmatoxylin and eosin. Differential staining is further discussed by Jacobs (1953).

Distribution

Toxoplasma appears to be ubiquitous in distribution and to be a parasite without specificity in warm-blooded animals.

Life-history

Toxoplasma is essentially an intracellular parasite with a predilection for the reticulo-endothelial and central nervous systems. Division is by longitudinal fission and a series of divisions may result in a mass of parasites within a single host cell. Eventually the aggregation of parasites becomes enclosed by a tough protective membrane and is then called a "pseudocyst". Schizogony, according to Jacobs (1953) has not yet been observed. Goldman *et al.* (1958) have described reproduction by internal budding.

Pathogenicity and pathology

There is a wide variation in host response and in strain virulence. Some strains appear to be avirulent but virulence may often be enhanced during passage through laboratory animals. The most important general clinical manifestations are encephalitis, a febrile exanthema with pneumonitis and enterocolitis. (Hoare, 1956). With a laboratory-passaged virulent strain in mice death may occur within a few days as the result of a fulminating infection which involves the brain. Following rapid proliferation, which affects all the viscera there is a copious

peritoneal exudate containing many free parasites. Encephalitis is characteristic of more chronic infection. In *dogs* infection seems to be widespread, Campbell *et al.* (1955 and 1958), Cole *et al.* (1953 and 1954). Lainson (1956) reported that 42 per cent. of 113 dogs from London possessed complement fixing antibodies against *Toxoplasma*. It seems probable that clinical disease is usually evident in young animals only (Jacobs *et al.*, 1955).

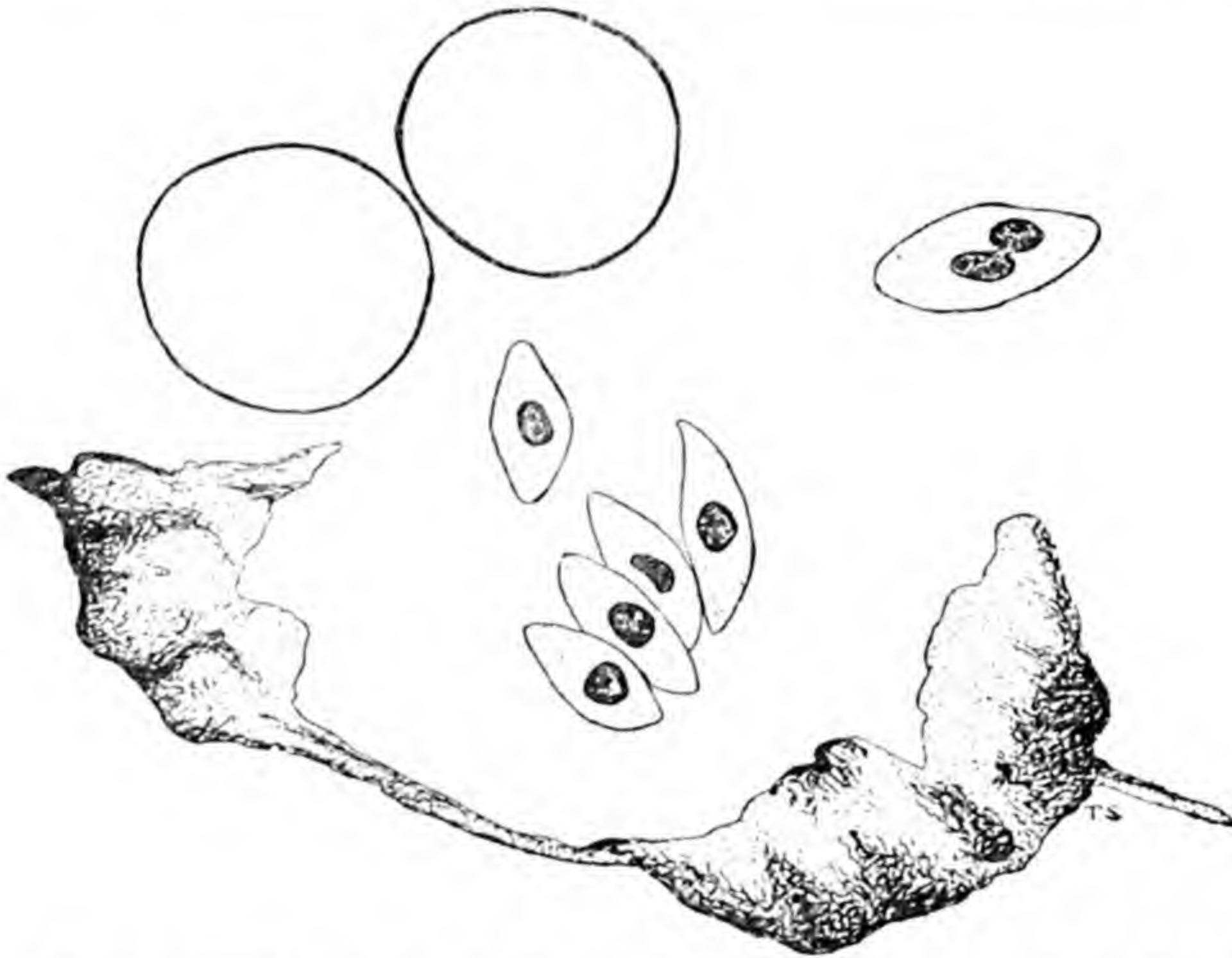


FIG. 34.—Toxoplasms from liver smear of rabbit with nuclear debris and two blood corpuscles.

The usual symptoms of clinical disease are gradual emaciation and enlargement of the lymph nodes. Dyspnoea is a common symptom and there is often a bloody diarrhoea. Occasionally the enlarged mesenteric lymph glands are inflamed with œdema and hæmorrhage of the adjacent tissues. There are nodules in the lungs and intestine and necrotic areas in the liver and spleen. There is eosinophilic infiltration where the organisms are found. When the host cell dies the parasites are released. Hartley *et al.* (1958) and Koestner and Cole (1960) have described meningo-encephalo-myelitis.

In *cats* the disease seems to be similar to that seen in dogs. Fourteen cases have been described by Meier *et al.* (1957). In acute cases there is persistent high fever and rapid pneumonic breathing.

In *pigs* an epizootic condition has been described in the U.S.A. the symptoms including debility, inco-ordination and enteritis with diarrhoea. Necrotic foci were present in the liver and brain. In Britain cases have been reported by Harding *et al.* (1961). The principal signs of illness were dyspnoea and wasting in piglets. Lesions and organisms were seen in the lung, liver, kidneys and lymph nodes of the piglets and *Toxoplasma* was recovered after mouse inoculation with material from the brain of the piglets' mother. Weinman and Chandler (1956) showed that 42 out of 88 pigs gave sera positive to the dye-test at a titre of 1:64 or more. There was considered to be an association between the infection in man and in pigs.

In *cattle* Sanger *et al.* (1953) reported disease of an acute, sometimes fatal character with fever, dyspnoea, extreme weakness and central nervous disorders. There were necrotic foci and calcifications in the brain tissue. There was evidence of congenital infection.

In *poultry*, a natural outbreak in fowls has been reported and the parasite was transmitted to mice (Erickson and Harboe, 1953).

In *sheep* a considerable number of investigations have been made since Olafson and Monlux (1942) reported a case of *Toxoplasma* encephalomyelitis in a sheep from New York which showed nervous symptoms for several days. At autopsy there were no gross lesions but in the brain there was an encephalomyelitis with slight meningitis. The cervical and thoracic regions of the spinal cord showed severe lesions, these consisting of marked monocyte perivascular infiltration. Cyst-like structures with the typical morphology of *Toxoplasma* were present in the inflamed areas. More recently particular attention has been paid to the association of the parasite with prenatal mortality (Hartley and Marshall, 1957 ; Rawal, 1959 ; Beverley and Watson, 1959 and 1961).

Diagnosis

Identification of the parasite is best attained by spinning fresh spinal fluid in a centrifuge, drying the sediment rapidly and fixing and staining. A complement fixation test (*e.g.* that described by Cooney, Kimball and Bauer, 1958) and the dye test described by Sabin and Feldman and developed by Beverley and Beattie (1952)

are available. Jacobs and Lunde (1957) described a hæmagglutination test and Jacobs and Melton (1957) a procedure for testing meat samples for the presence of *Toxoplasma*.

Transmission and epidemiology

The factors affecting transmission remain in doubt. Several attempts to identify an arthropod vector have not given consistent results although the organism can be shown to have very little resistance to environmental conditions outside the body. It seems that domestic animals, particularly dogs, play an important part in the transmission of the disease to man. There is a high rate of infection among veterinary surgeons (as evinced by serological tests) and people who keep kennels for dogs, and a considerable incidence of infection has been shown in pigeons and fowls. Uterine infection in women may lead to the death of the child and there is undoubtedly congenital transmission of infection in many other species.

Treatment

Aureomycin has been shown to check the disease if treatment starts at the beginning of infection but has little or no effect if given during the invasive phase. Sulphamezathine may assist clinical recovery. The disease responds markedly to treatment with sulphonamides and pyrimethamine in synergic combination.

Resistance and immunity

Reference has already been made to the wide variation in strain virulence which can be shown to occur. There is, however, no clear evidence of innate immunity in any species of warm-blooded animal. Some degree of resistance to reinfection is, however, characteristic of all the hosts so far studied. In cases of chronic infection the parasites often appear as agglomerations, known as pseudocysts, within the remnants of parasitised cells. Viable parasites are harboured for a very long time.

ENCEPHALITOZOON

The two genera *Toxoplasma* and *Encephalitozoon* closely resemble each other and it has been suggested that they are identical. *Encephalitozoon* was first described by Wright and

Craighead (1922) from the brain and kidney of a rabbit having a motor paralysis. Levaditi *et al.* (1923) named the parasite *Encephalitozoon cuniculi*. Encephalitozoon-like bodies have been reported from the spinal fluid and urine of man. (Matsubayashi *et al.*, 1959). There were pronounced clinical symptoms. According to Yost (1958) infection is seen in rabbits, mice, guinea-pigs, rats and occasionally dogs.

Differentiation from *Toxoplasma*

(1) The Sabin-Feldman dye test and complement fixation test for toxoplasms are negative.

(2) The organisms are usually smaller than *Toxoplasma*, appearing as uninucleate rods with rounded ends with homogeneous protoplasm. Extra-cellular forms are about $2.4-3.4\ \mu$ by $1.8-2.8\ \mu$ while intra-cellular forms are $1.5-3.0\ \mu$ by $1.4-2.8\ \mu$.

(3) The cytoplasm stains uniformly light blue with occasional pale areas with Giemsa, with a deep purple or red nucleus. *Toxoplasma* has granulated cytoplasm. With Goodpasture's stain encephalitozoa are deep blue, whilst in *Toxoplasma* the nucleus is coloured brown and the cytoplasm yellow so that the organisms are not easy to differentiate from the background tissue which stains yellow.

(4) *Encephalitozoon* is Gram+ ; *Toxoplasma* is Gram—.

(5) *Encephalitozoon* when inoculated experimentally into mice does not kill them and the virulence is not increased by repeated serial passage. *Toxoplasma* is usually fatal to mice at least on serial passage.

(6) *Toxoplasma* grows readily in tissue culture of monkey kidney even in the presence of penicillin and streptomycin. *Encephalitozoon* does not do so and in fact attempts to grow it on artificial media are not successful (Malherbe and Munday, 1958).

Distribution

Both *Toxoplasma* and *Encephalitozoon* may be found free in the tissues or in compact cyst-like accumulations. *Encephalitozoon* can usually be seen in the epithelial cells of the papillæ of the kidney, in the urine of affected animals and in the granulomatous lesions of the brain. (Yost, 1958). Here organisms may be found

singly or in clumps in granulomatous foci, in pseudocysts and in nerve tissue with no associated inflammatory reaction.

Pathogenicity

In laboratory animals the infection is usually evidenced only by a mild febrile disease. There may be an encephalitis. Lesions are mostly in the brain and kidney. According to Malherbe and Munday (1958) the infection is usually chronic ; motor paralysis may occur with swaying of the head from side to side.

Transmission

Although toxoplasms are readily transmissible by inoculation to a wide variety of hosts, it has been claimed that encephalitozoa are not inoculable, but Perrin (1943) found encephalitozoa of mice transmissible to mice, rats and rabbits by intracerebral and intraperitoneal inoculation, or by intranasal instillation. He did not succeed in infecting guinea-pigs. Levaditi *et al.* (1924) showed that transmission could be effected through urine.

Fragility of the organisms

Encephalitozoon will remain infective at 4° C. in either buffered glycerol or in Tyrode's solution, whilst toxoplasms will survive in Tyrode, but not in glycerol. Encephalitozoa will survive rapid freezing and storing for some weeks at -70° C., but toxoplasms will not.

Life-history

Ray (1941) describes an infection of a rabbit encountered in India. He describes multiplication by binary fission, and also a form of schizogony occurring in the epithelial cells of the urinary tubules. He also describes sporoblasts, and spores with a single nucleus, sporoplasm and a vacuolated area resembling a polar capsule. No polar filament was detected.

The complete life-history remains to be determined.

CHAPTER XIII

CILIATA AND PARASITES OF UNCERTAIN CLASSIFICATION

THE CILIATA

THE Ciliata possess cilia which serve as organs of locomotion. In most respects they are the most highly organised of all the protozoa. They are characterised by the possession of two nuclei ; a large *macronucleus* and a small *micronucleus*. A *cytostome*, which in its simplest form is represented by a small opening in the pellicle, may open into a gullet or *cytopharynx* through which food passes to the endoplasm. An oral groove (*peristome*) may lead to the cytostome. Special areas of cilia assist the collection of food. Sexual reproduction is mainly by conjugation.

Balantidium is the only genus of veterinary importance.

Genus *BALANTIDIUM*

Members of this genus are oval, ellipsoidal to subcylindrical organisms in which the peristome begins at or near the anterior end. The cytopharynx is not well developed ; the longitudinal ciliation is uniform ; there is a micronucleus and the macronucleus is elongated. The contractile vacuole is terminal. There is a number of species found in the gut of vertebrates. One of these may be pathogenic in domestic animals.

BALANTIDIUM COLI (Malmsten, 1857)

This species inhabits the large intestine of man, monkeys and pigs. Similar organisms may be found in other species of domestic animals.

Morphology

With the characters of the genus, *B. coli* is ovoid in shape and usually $50\ \mu$ to $60\ \mu$ in length, but may be considerably larger. The body is covered with a number of slightly oblique longitudinal rows of cilia. The small peristome is near the anterior

end and leads to the inconspicuous cytostome. There is a sausage-shaped macronucleus which lies transversely across the body. The small spherical micronucleus lies in a notch of the macronucleus. There are two contractile vacuoles and an anal opening. Food particles of various kinds are usually present. The parasites may be found as actively motile organisms in fresh fæces.

Life-history

This ciliate lives in the colon and cæcum of man and in the gut of the pig, where it feeds on all sorts of debris including erythrocytes and other host-cell fragments, starch grains, and fæcal material. It is only sometimes invasive but deep seated ulcers may occur in man.

Reproduction is ordinarily by transverse fission. At intervals resistant cysts, circular or ovoid in outline, are formed. These are slightly yellow or green in colour and measure $40\ \mu$ to $60\ \mu$ in diameter. Within the cyst the body of the organism can be seen with a contractile vacuole and a macronucleus.

Pathogenicity

The parasite is not usually markedly pathogenic in the pig and usually confines itself to the lumen of the gut. It may, however, take on an invasive phase if the gut wall has been first damaged with *Salmonella* infection. Infection in the pig is, however, of importance in its relation to man. In man, the ciliate lives in the colon and cæcum and may cause dysentery with invasion of the tissues of the mucosa and submucosa. Deep seated ulcers may eventually result. Infection is by ingestion of the resistant cysts, usually through contamination from pigs.

Kennedy and Stewart (1957) describe a clinical case in a five-year-old child whose infection was almost certainly acquired by eating dirt mixed with pig fæces containing *Balantidium coli*. Kennedy and his colleague make the point that in diagnosis it is important to look for the trophozoites and that fresh fæces are essential.

Balantidium in sheep and cattle

Hegner (1924) reported finding *Balantidium* sp. in the gut of Maryland sheep. The vegetative phase observed measured $45\ \mu$ by $33\ \mu$. There was no evidence of pathogenicity.

Dewes (1959) reported finding *Balantidium coli* in the faeces of a calf which showed tenesmus and passed bright blood after faeces of a consistency softer than usual. The calf was grazing on land used by pigs.

Treatment

In the treatment of their human case Kennedy and Stewart (1957) found that Stovarsol appeared to eliminate the parasites.

PARASITES OF UNCERTAIN CLASSIFICATION ANAPLASMA-EPERYTHROZON-HAEMOBARTONELLA GRAHAMELLA-SARCOCYSTIS-GLOBIDIUM

These are parasites none of which appears to be closely related to the protozoa of the main classification. Until recently, *Sarcocystis* was included in an Order *Sarcosporidia* Balbiani, of the *Sporozoa* but the work initiated by Spindler and Zimmerman (1945) indicates that *Sarcocystis* is more closely related to the fungi.

ANAPLASMA MARGINALE Theiler, 1910

A. marginale is the parasite involved in the disease of cattle known in Africa as *gall-sickness*.

Morphology

In their original studies on Texas fever (*Babesia bigemina*) in the U.S.A., Smith and Kilborne noted some "marginal dots" on the edges of the red blood corpuscles. Anaplasma bodies do, in fact, appear as spherical granules which stain bright red with Wright's stain. As a rule there are only one or two organisms in each cell and there is usually no apparent structure. No cytoplasm is apparent but a faint halo (probably an artefact) is often observed round the granule which usually measures from $0.1\ \mu$ to $0.5\ \mu$ in diameter. Lotze and Viengst (1942), after examination with the ultra-microscope, concluded that Anaplasma occurs in three forms:—

(1) As extra-erythrocytic bodies $1.0\ \mu$ long by $0.1\ \mu$ to $0.2\ \mu$ in diameter with a knob-like body at one end.

(2) Smooth anaplasms, $0.2\ \mu$ to $0.5\ \mu$ in diameter, inside the red cells.

(3) Rough anaplasms, $0.6\ \mu$ to $0.9\ \mu$ in diameter each containing eight spore-like bodies, also intra-erythrocytic.

More recently a good deal of work on the morphology of *Anaplasma* has been recorded. Franklin and Redmond (1958) have described the appearance of projections or "tails" extending from typical *Anaplasma* bodies. These were considered to indicate a stage in normal development of *Anaplasma marginale* in the blood of cattle. Ristic (1960) as the result of electron-microscope studies on *A. marginale* came to the conclusion that three morphological forms do indeed exist and that they are referable to:

(a) the classic marginal inclusion ($0.3\ \mu$ to $0.1\ \mu$)

(b) a smaller form (initial body) which is part of (a) and which measures 300-400 m μ in diameter

(c) a still smaller form (part of the initial body) known as a polyhedral body and measuring about 10 to 20 m μ in diameter. Form (a) may include 1-8 of the (b) forms which may occur individually or two together in an erythrocyte.

Persistence of these initial bodies in the erythrocytes of carrier animals probably indicates that it is the form concerned with the survival of the parasite in the premune state.

Distribution

The parasite is found widely throughout the tropics, throughout Africa, the warmer parts of America, the Middle East, some parts of Southern Europe and the Far East.

Life-history

The life-cycle is not completely known. Binary fission is believed to be the usual means of multiplication but reference has already been made to the possibility of multiple fission with the formation of eight small spherical bodies (Lotze, 1946) and the work of Ristic (1960).

Transmission

Very many different species of ticks have been implicated as vectors and it has been shown that transmission readily occurs through the medium of various mosquitoes and species of *Tabanus*. Uterine transmission has been reported and a very important means of spread is through the use of unsterilised surgical instruments, following mass inoculation campaigns where many animals

are injected with the same needle, and following other operations such as horn-tipping and castration.

Symptoms and pathology

The incubation period in experimental animals is 14-15 days : sometimes longer (Twiehaus, 1958). Disease may be peracute, acute or chronic, presumably depending on the age of the animal and its immunological state.

In the early stages of acute anaplasmosis a fever of 103° F. to 107° F. usually occurs. As the disease progresses the temperature may become normal or subnormal before the animal dies. Breathing is accelerated and heavy. Exhaustion, lack of rumination and loss of appetite are general symptoms. The skin and all visible mucous membranes become yellow and anæmic. There is a depraved appetite. Animals walk with a stiff unsteady gait. Urination is frequent but the urine is normal in colour.

Sometimes there is constipation with dark, blood-coloured fæces covered with mucus. Glands are enlarged and there is a rough coat with œdema round the eyes. Young animals are as a rule more resistant than are old ones. Acute cases may result in death within 24 hours. The average fatal case lingers for three or four days.

With chronic cases there is a severe anæmia with a very low red blood cell count and with hæmoglobin less than 10 per cent. of normal. Recovery is very slow and it may be many weeks before the patient fully recovers. Under field conditions in Africa the animal is likely eventually to succumb to a combination of factors including the usual stress factors such as chronic malnutrition, with which much of the native stock has to contend.

In late pregnancy, cows may abort. Recovered animals are believed to remain carriers for life but are resistant to further infection.

Post-mortem examination shows the lymph glands to be enlarged and œdematous. The heart is enlarged and covered with petechial hæmorrhages. The blood is thin and watery with all the characters of an extremely severe anæmia. Gastro-intestinal catarrh is usually present. The lungs are anæmic with some emphysema. The liver is saturated with bile and enlarged. The spleen is enlarged with a soft pulp.

Epidemiology

The disease is essentially one of new imports ; young indigenous stock tending to get a mild infection with the acquisition of the carrier state and some degree of resistance to reinfection. Latent *Anaplasma marginale* infection may persist in hosts other than cattle e.g. Columbia black-tailed deer (*Odocoileus hemionus columbianus*) which were shown by Christensen *et al.* (1960) to be carriers 11 months after they were infected with blood from bovine carriers.

Anaplasma may be transmitted through the egg of the tick from one generation to the next and may remain in the ticks for as long as 4-5 years from the time the initial tick fed on an infected animal. (Anon, 1958).

Diagnosis

Diagnosis is usually on the presence of the marginal bodies in the red blood cells. It is possible for confusion to occur when examining the blood of young or anæmic animals in which *Jolly bodies* occur. These are probably degenerate nuclei of immature red blood cells. Boynton and Woods (1935) reported a nonspecific serum-precipitation test which is of some value.

A complement fixation test has recently found wide use in the U.S.A. (Anon, 1958). It is used to identify carrier animals which are subsequently slaughtered. It is considered that the disease can be eliminated subject to :

- (a) adequate general application of the control measures
- (b) that transmission is through mechanical vectors and not ticks
- (c) that insect vectors are controlled by sprays
- (d) that all hygienic precautions are taken by veterinarians and ranchers.

The complement-fixation test is described in detail and standards are laid down in the report of the Proceedings of the U.S. Live-Stock Sanitary Association, held in St. Louis, Missouri in 1957. The antigen consists of the antigenic fraction of bovine lysed erythrocytes collected from an acute experimental infection. Clear phenolised non-hæmolysed serum of a suspected animal is tested after incubation at 58° C. for 35 minutes.

The hæmolytic system is:

(a) complement (normal guinea-pig serum)

(b) washed sheep red blood cells and hæmolytic amboceptor (anti-sheep hæmolysin)—which are mixed in equal parts. The test does not seem to be 100 per cent. accurate and there is some evidence of interference between *Anaplasma* and *Eperythrozoon*.

Ristic, White and Sanders (1957) have described the use of fluorescein-labelled antibody to detect *Anaplasma* bodies particularly when they occur sparsely in the blood of carrier animals. The basis of the test involves the immunochemical properties of the antigen-antibody complex wherein the combined fluorescent antibody renders the organism visible under the fluorescent microscope.

A globulin fraction was separated from the sera of calves experimentally infected with *A. marginale* and conjugated to fluorescein. Alcohol-fixed organisms present in an infected blood film became fluorescent when exposed to the conjugated globulin. The ability of an unlabelled immune serum to block the fluorescent reaction offers a means of testing unknown sera for the presence of antibody.

Treatment and control

Until comparatively recently no treatment seemed to have any specific effect against *Anaplasma marginale* although symptomatic treatment and careful nursing appeared to have a marked effect in reducing mortality under tropical conditions. Untreated cases often recover.

A significant effect using paludrine, quinoline diphosphate or Aralen diphosphate has, however, been reported, and Foote, Farley and Gallagher (1951) have reported on the use of aureomycin against anaplasmosis in America. The drug was given initially at the rate of 5 mg. per lb. body weight followed by 2.5 mg. per lb. at 12-hourly intervals for five days. It appeared to prevent the development of clinical symptoms if given at the time of infection but was of little effect once the disease was established.

Several workers have confirmed the effects of the tetracycline group of antibiotics on *Anaplasma* e.g. Miller *et al.* (1952) with oxytetracycline, but under field conditions such drugs are usually too expensive for general use. Staley *et al.* (1959) screened five

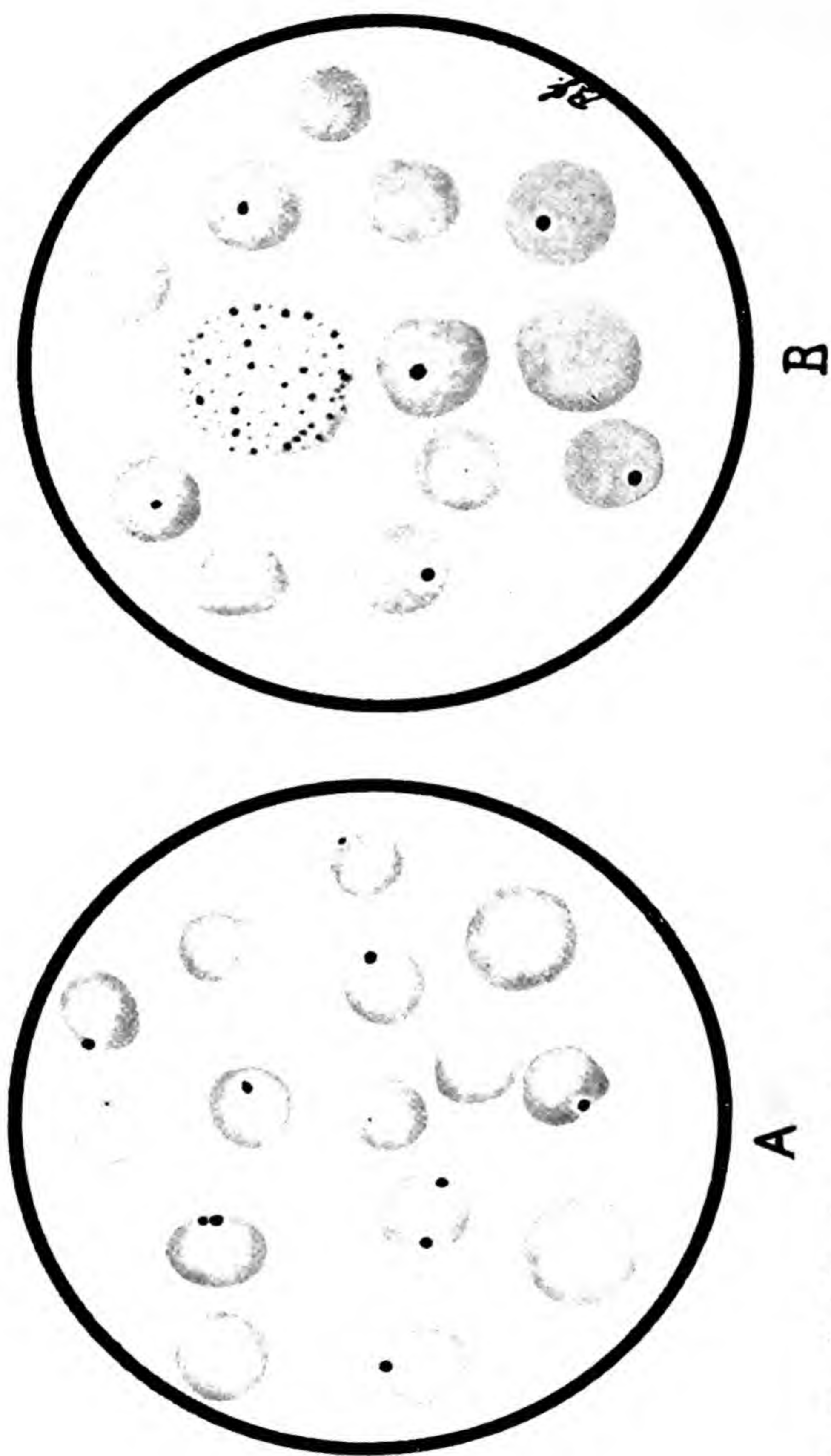


FIG. 35.—Red blood corpuscles of cattle showing infection with *Anaplasma marginale* (A) and *Anaplasma centrale* (B). In B an enlarged red blood corpuscle shows punctate basophilia. (After Wenyon, 1926)

drugs and obtained some evidence of activity particularly with a quinolinium derivative. Blood transfusions assist clinical recovery.

Stock can satisfactorily be protected against the clinical disease by inoculation, preferably when young, with the relatively benign *Anaplasma centrale*.

ANAPLASMA CENTRALE Theiler, 1911

This parasite is morphologically very similar to *A. marginale* from which it is differentiated, as its name suggests, by its central position in the red blood corpuscle, and by its relatively benign effect on cattle.

ANAPLASMOSIS IN ANIMALS OTHER THAN CATTLE

A. marginale can be maintained indefinitely by passage through sheep and goats to which it is not pathogenic, but the parasite appears also to lose virulence to cattle. Ovine passage is a convenient way of separating *Anaplasma* from other parasites such as *Babesia bigemina* which does not survive except in its natural hosts.

Anaplasmosis has been described from sheep, goats, donkeys, swine, dogs and horses but little is known of the importance of these conditions. In South African sheep the disease is reported to be mild but in Palestine it may be severe and cause serious mortality. Infection in swine was reported by Doyle (1934) in America and Cerruti (1939) in Italy.

EPERYTHROZON, HÆMOBARTONELLA (BARTONELLA) AND GRAHAMELLA

The precise systematic position of these organisms is in doubt. As Neitz (1934) however, has suggested they appear to have affinities both with one another and with *Anaplasma*.

EPERYTHROZON

Morphology

These are pleomorphic organisms found in blood, occurring on the surface of the erythrocytes or in the plasma between them. They usually appear as minute rings 0.5μ to a maximum of 1.0μ in diameter. Occasionally larger forms have been recorded ;

E. suis can sometimes appear as discs $2.5\ \mu$ in diameter. The organisms stain well with Giemsa and other Romanowsky stains.

Distribution and species specificity

Different species of *Eperythrozoon* have been reported from a variety of hosts throughout the world. Horses and poultry are apparently not affected (Weinman, 1944). *E. wenyon* was reported from cattle by Adler and Ellenbogen, 1934 ; *E. coccoides* from mice by Schilling (1928), McCluskie and Niven (1934) and

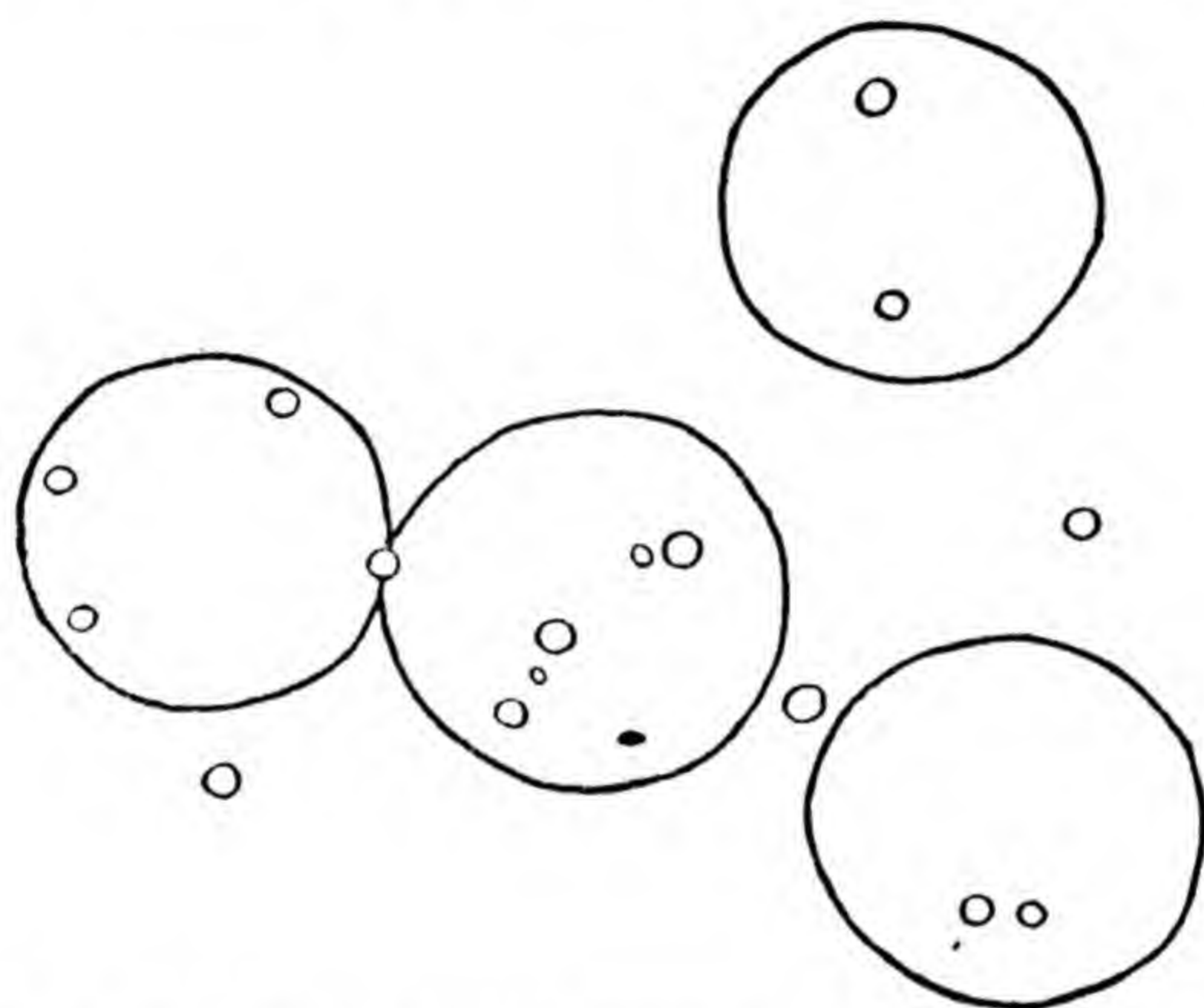


FIG. 36.—*Eperythrozoon coccoides* in blood of mouse.

Derrick *et al.*, 1954 ; *E. ovis* from sheep by Neitz (1934 and 1937) and Littlejohns (1960) ; *E. parvum* and *E. suis* from pigs by Splitter (1950), Jennings and Seamer (1956), and Savage and Isa (1958) ; *E. felis* from cats, Seamer and Douglas (1959).

The parasites found in domestic animals generally seem to be host specific.

Life history and transmission

Details of the life-history are unknown. As shown by Thurston (1955), the parasite can be transmitted in citrated blood injected intraperitoneally or intravenously or given by the mouth. The same blood failed to set up an infection when smeared on the external mucous membranes. Urine and faeces were not infective when injected intraperitoneally. *Eperythrozoon* in citrated blood was not resistant to drying or to 0.5 per cent. phenol. It remained

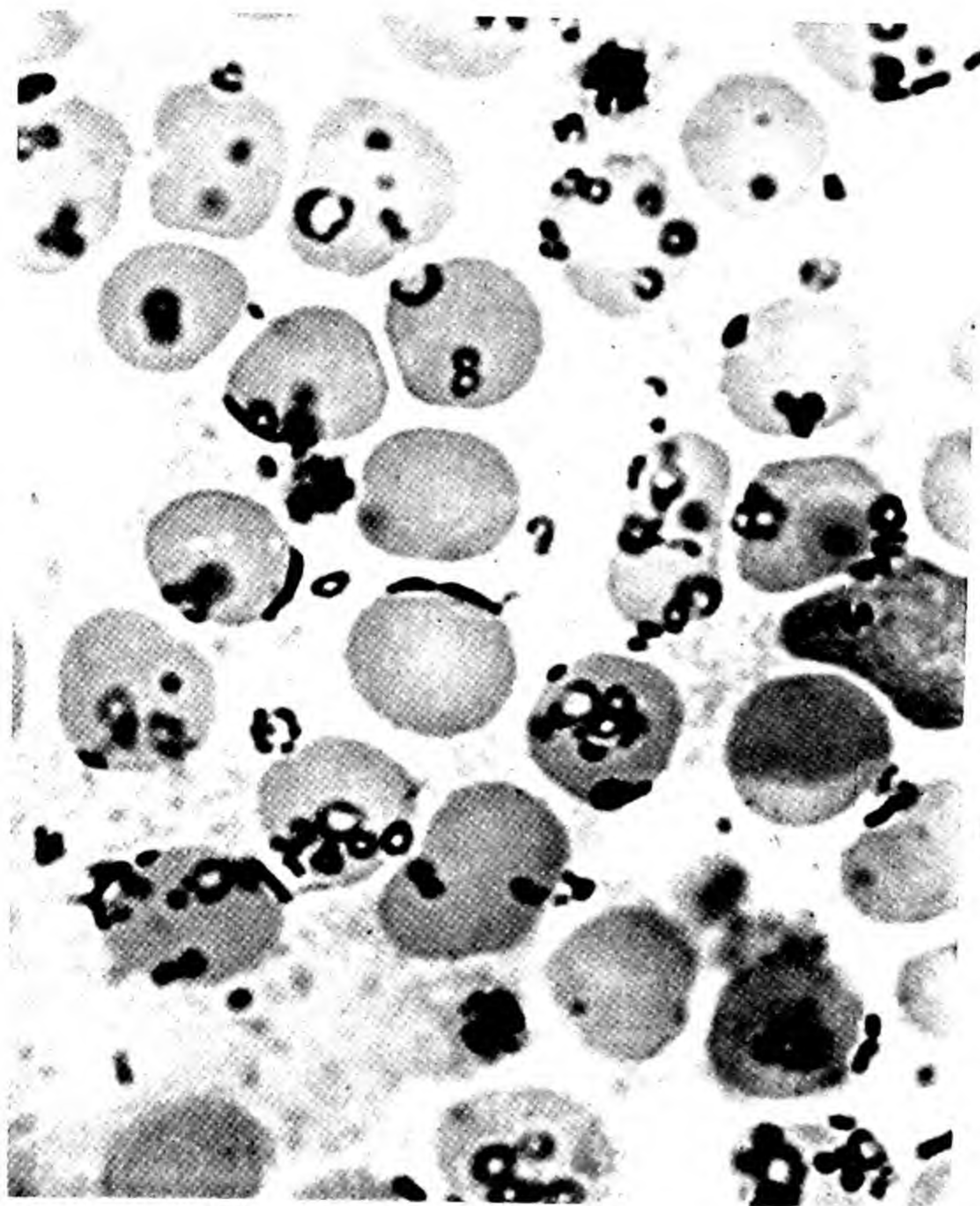


FIG. 37.—*Eperythrozoon suis*

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infective for eleven days at 3° C. and resisted lysis of the erythrocytes but was killed in three hours at 37° C. *E. parvum* and *E. coccoides* have been shown by Eliot (1936) and Jansen (1952) to be transmitted by lice.

Pathogenicity

Species of *Eperythrozoon* seem usually to be benign parasites. Fulminating blood infections with rise of temperature, icterus and anæmia can occur. Evident pathogenicity has been reported in, for example, laboratory colonies of mice, in cattle and in sheep, Neitz (1934 and 1937). Splitter (1950) has described a disease of pigs (icteroanæmia) which resembles bovine anaplasmosis. In general the disease is seen only in splenectomised animals.

Immunity, epidemiology and diagnosis

Infection is probably very widespread with the parasite in a state of premunition which may be upset by stress factors *e.g.* concurrent infection with *Plasmodium berghei* (Thurston, 1955) and as indicated, by splenectomy. A complement fixation test for diagnosis of the condition in pigs has been described by Splitter (1958).

Treatment and control

The disease is not usually of great importance but the parasites respond to treatment with organic arsenical compounds *e.g.* neoarsphenamine (0.3 mg./20 gm. mouse) and also to tetracycline antibiotics (Splitter and Castro, 1957) and to organic antimonial compounds.

HAEMOBARTONELLA

(*Bartonella* is considered to be synonymous with *Hæmobartonella*.) *Hæmobartonella* is similar in size and staining reaction to *Eperythrozoon* from which, however, it differs in appearing, usually, as small cocci or beaded bacilli which are within the corpuscle not on the outside. Several species have been recorded in different hosts ; in rats and mice (Mayer, 1921 ; Bayon, 1928 ; Neitz, 1938) ; in cats (Flint *et al.*, 1958) ; in the dog (Kikuth, 1928 ; Donavon and Loeb, 1960). *H. muris* from the rat is transmissible to mice (Neitz, 1938).

Pathogenicity

The parasite is usually relatively non-pathogenic. Symptoms most often follow splenectomy and are characteristically those of an anæmia. Flint, Roepke and Jansen (1959) described anæmia in cats following the injection of blood infected with *H. felis* either intraperitoneally or intravenously or by oral inoculation. In this instance splenectomy apparently had little effect on susceptibility to infection. Resistance to reinfection was not very evident.

Transmission

Kessler (1942) has recorded the preservation of *Bartonella muris* in the frozen state. Crystal (1958) has investigated the mechanism of transmission of *H. muris* to rats through the spined rat louse *Polyplax spinulosa*. Balazs *et al.* (1961) apparently showed development of *H. felis* in culture.

Control

Hæmobartonella responds to the same chemotherapeutic measures as does *Eperythrozoon*.

GRAHAMELLA

This organism is another member of the *Eperythrozoon-Bartonella* group, occurring as fairly regular rods which are intracellular, in the erythrocyte. It appears to be non-pathogenic. Neitz (1938) has reported the occurrence of parasites in rodents and Carpano (1935), in fowls.

SARCOCYSTIS

Information on sarcosporidiosis has been reviewed by Teichmann (1912) by Scott (1930 and 1943, *a* and *b*) and by Eisenstein and Innes (1956). These last authors make the point that practically nothing has been added to our knowledge of the group since Scott's review of 1930. The exception is the work of Spindler and Zimmerman (1945) whose investigations have suggested that the genus *Sarcocystis* should be separated from the *Protozoa* on the grounds of its affinities with fungi. Aseptically ruptured cysts of *Sarcocystis miescheriana* were placed in sterile dextrose solution in which development of the parasite was

followed. In a period of from a few days to a few weeks the "spores" budded off minute coccoid bodies which developed into septate mycelia with vertical hyphæ bearing spores, this being typical of the type of development seen among fungi of the *Aspergillus* group. Further work by these workers tended to confirm their view of the nature of *Sarcocystis*.

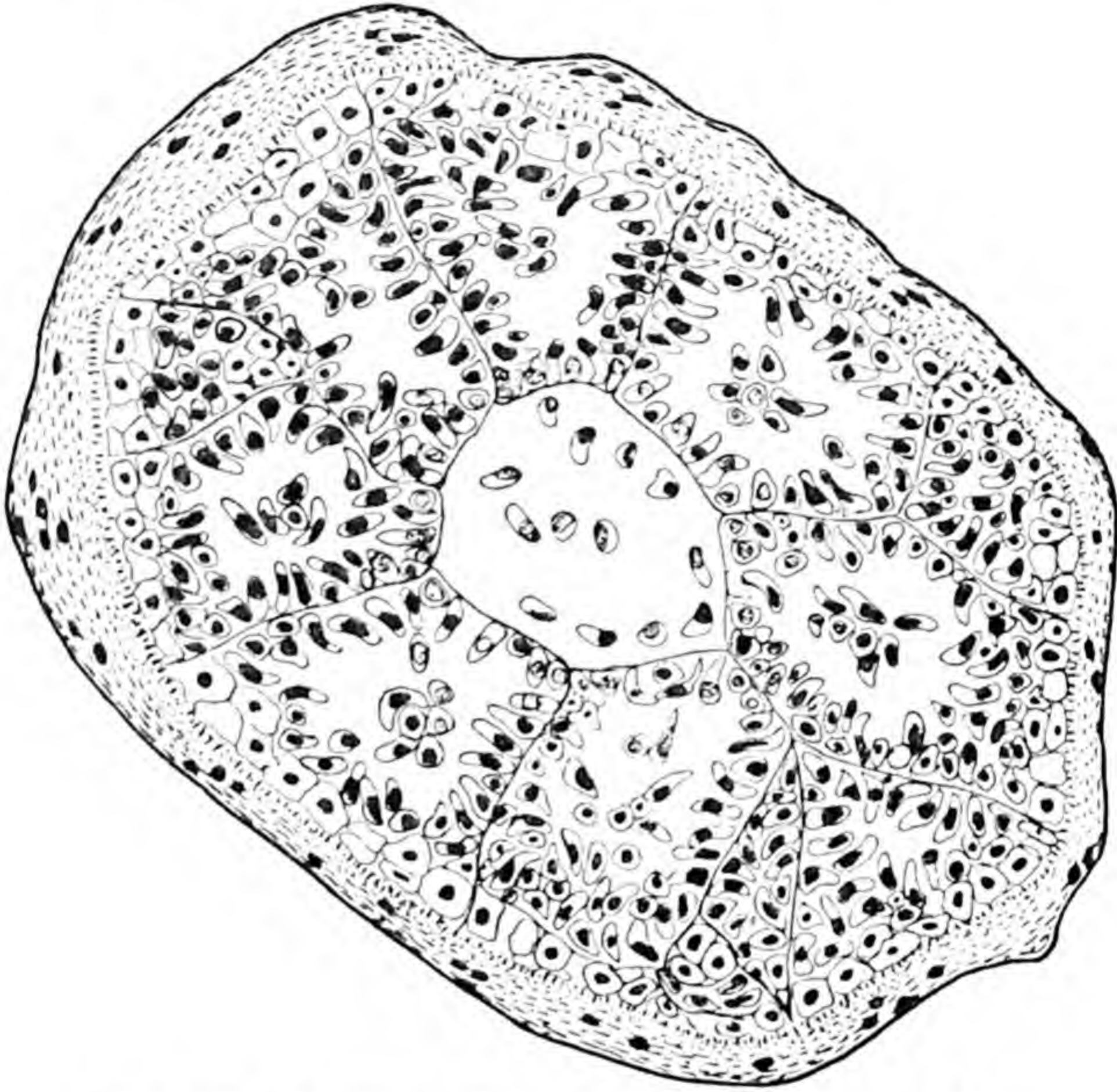


FIG. 38.—Section of sarcocyst from tongue of ox.

This work has not so far, however, been confirmed and it is worthy of note that viable spores of *Sarcocystis*, isolated from muscle, are motile under certain conditions. This motility would seem to preclude their being closely related to *Aspergillus*.

Morphology of the parasite

The organisms are primarily parasites of striated muscle and usually occur in herbivores. They occasionally infest unstriated muscle and have been recorded in the dura mater of the brain (Scott, 1930) and in blood (Hall, 1936). In some instances the presence of organisms in blood smears may be due to contamination from the skin, but Scott (1943*a*) accepts their presence

as indicating disintegration of the sarcocyst during normal development. Sarcosporidia have also been reported from omnivorous mammals and reptiles. Infected host muscles are characterised by the presence of opaque white bodies, which may vary in size from microscopic proportions up to several centimetres in length and which are called *Miescher's tubes*. These are cylindrical, ellipsoidal or ovoid in shape with a lobulated surface. Although the forms occurring in different species of host may vary in shape and size and have as a result been given different specific names, it is highly probable that all belong to a single species, the variations resulting from different host-parasite reactions.

The *Miescher's tubes* are enclosed in a membrane which divides the mass of the parasite into a number of chambers by a series of septa. When mature the parasite becomes filled with "spores" or *Rainey's corpuscles* which are crescentic or sickle shaped, about $10\ \mu$ to $12\ \mu$ in length by $4\ \mu$ to $9\ \mu$ in breadth. They are covered with a delicate membrane and contain a nucleus and many granules.

Life-cycle

It is believed that infection is by ingestion and it has been shown that mice can be infected by feeding on infected sheep muscle or by feeding on the faeces of infected animals. Resistant forms of the parasite have been described in the intestines of mice and these may be the forms which are passed out in the faeces. Very young animals do not appear to become infected at all readily. There is no evidence of the intervention of an insect vector. Spindler (1946) has described the excretion of an infective stage by animals which had consumed the infected flesh of swine.

The sarcocyst is first seen as a one-celled amœboid parasite within a striated muscle cell. By growth and repeated division a young sarcocyst, consisting of a number of rounded nucleated cells (sporoblasts) develops and the whole becomes enclosed in a cyst wall, the outer part of which is probably derived from the host while the inner part and the septa are formed by the parasite. The sporoblasts are transformed into ellipsoid and then into banana-shaped spores. The cysts lie within a single muscle fibre parallel to the long axis squeezing aside the striated sarcoplasm.

If the parasite grows larger than the muscle cell it may come to occupy the interfibrillary tissue. In old large sarcocysts the centrally located spores may degenerate and disappear. The cyst walls of other sarcocysts eventually rupture and the spores presumably enter the blood stream. Their subsequent fate is uncertain. It has been shown experimentally that the faeces of an infected animal may contain organisms in a form that is infective to other animals. No intermediate host seems necessary for transmission of the disease. Ingestion is almost certainly the normal route of infection (Spindler, 1946).

Pathogenicity

Sarcosporidiosis is of economic importance primarily because infestation may lead to condemnation of meat intended for human consumption. Gross infection can apparently lead to pathogenicity but the parasite usually seems to be benign and there is rarely marked tissue reaction to its presence. Sarcocysts contain a toxin "sarcocystin" which is fatal in a few hours if injected into mice or rabbits.

Epidemiology

Neither life-history nor epidemiology are adequately understood. A seasonal variation in the rate of infection with greater severity in spring and summer has been reported by Scott (1919).

Geographical distribution and incidence

Sarcosporidia appear to have a ubiquitous distribution. The incidence of infection is very variable but may be exceedingly high in, for example, sheep. Man is apparently rarely infected.

Species

A wide range of so-called species has been described :—

Sarcocystis tenella (Railliet, 1886) of sheep occurs principally in the œsophagus, but may occur in the diaphragm, heart, etc. The parasite is large and easily visible to the naked eye, being about 1 cm. in diameter. Brownlee (1936) recorded the detection of a microscopic sarcocyst in the brain of a sheep.

Sarcocystis hirsuta (Moule, 1887) of the ox occurs in the tongue and heart muscle and may reach the size of a pin's head.

Sarcocystis bertrami (Doflein, 1901) of the horse, ass and mule occurs in the diaphragm and other muscles and is microscopic.

Sarcocystis miescheriana (Kuhn, 1865) of the pig occurs in the tongue and heart muscle and is just visible to the naked eye.

Sarcocystis rileyi (Stiles, 1893) of the duck, is 4 to 8 mm. in length by 2 mm. in diameter and occurs throughout the skeletal muscles.

Sarcocystis horvathi (von Ratz, 1908) of the fowl occurs in the muscles of the head, neck and pelvis.

GLOBIDIUM Flesch, 1883

The status of the genus has been reviewed by Pellérdy (1960) and by Pals (1960).

As indicated by Richardson and Kendall (1957) species of *Globidium* were considered to be responsible for two different syndromes—a serious skin condition (cutaneous globidiosis), characterised by warm painful swellings, thickening of the skin, loss of hair, cracking and necrosis (Bennett, 1933 ; Hofmeyr, 1945 ; Pals, 1954), and an infection of the stomach and intestine—the intestinal globidiosis described by such authors as Triffitt (1925 and 1928), Soliman (1958 and 1960), Rac and Willson, (1959) Neuman and Nobel (1960).

It seems reasonable to accept Pals's (1960) view that cutaneous globidiosis is in fact caused by *Besnoitia besnoiti* (Marotel, 1912). The ætiology of the intestinal disease usually attributed to infection with *Globidium* is difficult to determine.

Globidiosis of the intestine

The condition usually found in sheep and goats is characterised by the presence of white round opalescent cysts, usually measuring 300-500 μ in diameter and with a wall 5-6 μ thick, embedded in the mucosa of the abomasum and the intestine. The cyst contains large numbers of spores, "banana" shaped, with a nucleus at the blunt end and a distinct granule towards the opposite sharp hyaline end and measuring between 4.5 μ and 10.0 μ by 1.2 μ to 1.8 μ . A cyst may contain also a nucleus which is apparently derived from the host cell. The pathogenicity of the parasite is in doubt although cases are reported in which infestation appears

to be associated with enteritis and severe diarrhoea with bloody faeces, loss of appetite and rapid emaciation. Examination of the literature dealing with intestinal globidiosis suggests that some, at least, of the authors may have been dealing with cases of coccidiosis caused by species of *Eimeria*. Some authors stress the fact that species of *Eimeria* were absent although it is not always easy to see in what way the various developmental forms described differ from those characteristically found in *Eimeria*. On the other hand, development in the abomasum seems to be characteristic of *Globidium* while it is certainly not characteristic of those species of *Eimeria* so far described in the sheep and goat.

In view of the apparently widespread distribution of the parasite—32 per cent. of sheep and 40 per cent. of goats examined in the Sudan (Soliman, 1960) ; 34 per cent. and 94 per cent. of sheep and goats in different areas of Pakistan (Sarwar, 1951) ; 92 per cent. of a group of sheep examined in England (Triffitt, 1925) it seems astonishing that more precise work on the life-history has not been recorded. In the existing state of knowledge it is probably advisable to consider *Globidium* to be a parasite with a somewhat dubious ancestry and with a distinct affinity to *Eimeria*. Such is certainly the view of Pellérdy (1960).

Cutaneous globidiosis (Besnoitiosis)

Pols (1960) has reviewed the status of *Besnoitia besnoiti*, showing that the parasite has at various times been allotted to three genera—*Sarcocystis*, *Gastrocystis* and *Globidium*. The genus *Besnoitia* is regarded as having affinities with *Toxoplasma* from which it is differentiated (Pols, 1960) by the fact that members of *Besnoitia* are harboured temporarily by monocytes and permanently by histiocytes while *Toxoplasma* spp. chiefly parasitise cells of the reticulo-endothelial system, macrophages, monocytes and endothelial cells. The species of veterinary interest cause serious skin disease in horses and cattle in Europe (France and Portugal) and in Africa and there are reports from Mexico. In Alaska a species has been identified in reindeer and caribou. In a suggested reclassification of the group Pols (1960) separates the parasite found in the horse—*B. bennetti* Babudieri (1931) from *B. besnoiti* (Marotel, 1912) in cattle. Apart from the fact that Pols was unable, in a very small trial, to transmit a strain

from cattle to a horse, there is not much evidence that the two species are in fact distinct.

Besnoitiosis in horses (B. bennetti).

Bennett (1927 and 1933) described a condition seen in the Sudan in horses and cattle and attributed by him to infection with *Globidium*. Animals from one particular area seemed to be affected. The disease in horses was primarily of the skin, sub-acute or chronic and often resulting in death. An animal might have a history of seven or eight months' illness with extreme weakness and dejection, thickened eyelids, swollen legs, loss of hair, scab formation and general thickening of the skin. The buccal mucous membranes were pale and dry. There could be considerable damage from bursting of the skin cysts. Cysts might be visible in the nostrils.

On post-mortem examination obvious abnormalities were essentially limited to the laryngeal region and the skeletal muscle. The muscles were markedly degenerated, possibly from some toxic action as there was no evidence of parasites. The mucous membrane of the whole of the larynx anterior to the glottis was, however, studded with many hundreds of spherical white cysts about 0.5 mm. in diameter. There was a predilection for stratified rather than ciliated epithelium. Daily blood examinations usually showed the presence of "sarcospores" *i.e.* the trophozoites of *Besnoitia*. The skin cysts which were the most characteristic form of the parasite were really in the matrix of the areolar or adipose tissue of the subcutis or in the intercellular spaces of the more highly differentiated layers.

Diagnosis

This is effected by deep skin scraping and demonstration of the parasite.

Besnoitiosis in cattle (B. besnoiti)

The disease is characterised by infection of the dermis, subcutaneous tissues and fascia, the connective tissue of the scleral conjunctiva and the laryngeal mucosa.

Initially, there is pyrexia with warm painful swellings on the limbs and dependent parts. In later stages the more characteristic

involvement of the skin is apparent ; hair falls out, the skin is thickened and may crack with exudation of blood followed by necrosis. Parasites are sometimes found in blood and tissue smears but appear most characteristically in white cysts (up to $600\ \mu$ in diameter) in the cutis and sub-cutis, the sclera and the mucous membranes of the upper respiratory tract.

Morphology of the parasite

The description of the morphology of the parasite given by Pols (1960) is based largely on observations of infection for a limited period during the developmental cycle in the rabbit. Trophozoites are seen either extracellular or intracellular in monocytes in the blood stream or in lung or testis smears and are oval, slightly pointed at one end, curved with rounded ends (banana shaped), crescentic, or rarely, round. They measure $5\ \mu$ to $9\ \mu$ in length by $2\ \mu$ to $5\ \mu$ in breadth. With Giemsa stain the cytoplasm is shown to be granular and stains blue. The reddish-purple nucleus is usually near the centre but is variable in position. Oval forms may be binucleate. Similar organisms may be found singly or in groups between connective tissue fibres or they may be in histiocytes where they occupy vacuoles in the cytoplasm. Fully developed cysts which can be as much as $600\ \mu$ in diameter in cattle are spherical or slightly spheroidal in shape but when present in very large numbers may be compressed so that the cyst walls become confluent.

Each cyst is packed with trophozoites—mostly crescentic and $2\ \mu$ to $7\ \mu$ in length. In saline suspension they exhibit moderate motility. The capsular zone apparently develops as a host reaction to the presence of the parasite.

Species of animal susceptible

B. besnoiti, according to Pols (1960), can be transmitted from naturally infected cattle to rabbits and back again, but not to guinea-pigs, rats or mice. Parasites established in the rabbit can be transmitted to sheep and goats and to a limited extent to guinea-pigs. Attempts to transmit from rabbits to horse or dog were not successful (Pols, 1960).

Further critical tests would be needed to confirm that *B. besnoiti* from cattle is not capable of infecting the horse.

Life-history

After the artificial infection of a susceptible host there is a febrile reaction during which parasites may appear in the blood stream, in superficial lymph nodes, in subcutaneous œdematous fluid and in lung, testis, liver and spleen. It is probable that extracellular parasites occurring in the blood invade monocytes and reproduce by binary fission. Very rarely multiple fission is observed. During the later stages of the febrile reaction there seems to be an increase in the banana-shaped and crescentic parasites which are subsequently seen in the interstices of the subcutaneous and cutaneous connective tissue where they enter tissue histiocytes. The parasites are then seen in vacuoles of the host cell which undergoes considerable reaction.

In the parasitised and hypertrophied histiocyte multiplication proceeds until the resulting cyst is filled to capacity.

Transmission

Until recently the method of transmission was unknown, direct contact between infected and uninfected animals was apparently not sufficient. Bigalke (1960) reports mechanical transmission of cysts of *Besnoitia besnoiti* from a chronically infected bull to rabbits by *Glossina brevipalpis*. Under field conditions there is a seasonal incidence of infection which suggests the possibility of an arthropod vector being involved. Parasites in cysts may apparently remain viable for as long as ten years. Under laboratory conditions, intravenous, subcutaneous or, preferably intraperitoneal injection of cardiac or ear-vein blood or serous fluid or spleen suspension from a rabbit in the febrile stage will set up an infection. It appears, however, that the period of infectivity of the blood, particularly in cattle, is short. Attempts to transmit the cutaneous parasites using ground skin tissue suspended in saline have not been successful.

Maintenance of the parasite

B. besnoiti survived in citrated blood for four days at 4° C. (Pols, 1960).

Pathogenicity, pathology and symptomatology

In cattle few animals ordinarily show clinical symptoms; the rate of mortality rarely if ever exceeds 10 per cent. Nevertheless

animals may lose condition, cows may abort, bulls become sterile and hides are valueless for tanning. In young animals there is probably mild infection followed by a condition of premunition. In rabbits there may be peracute disease with death in a few days. In cattle there can be an initial incubatory period of 6-10 days with pyrexia for 3-10 days and a temperature of 103° F. to 107° F., photophobia, œdematous swellings on limbs and pendulous regions and enlargement of the lymph nodes. Other cases may be afebrile and with no characteristic clinical picture. In severe cases the second (depilatory) phase is the critical one. Advanced skin lesions develop with folding and cracking and serous exudate with blood. Secondary bacterial invasion may be responsible for death. Severely affected cases may remain emaciated for months and the skin remains thickened and wrinkled for life although a certain amount of regrowth of hair may occur. At autopsy there is no characteristic change in the internal organs.

Diagnosis

Diagnosis depends on demonstration of the parasite. In the early stages the only available method with the living animal is transmission of blood to a rabbit. Later, skin biopsies may be taken or deep skin scrapings examined. At autopsy the cysts may be visible as opaque whitish raised nodules (0.5 mm. in diameter) in the mucous membranes of trachea, cutis and subcutis. There are never any cysts in the mucous membranes of the abomasum or large or small gut.

Immunity

It is probable that resistance to reinfection is manifested by a state of premunition.

Treatment and control

Leitao (1949) used injections of a 10 per cent. solution of formalin ; Herin (1952) a single intravenous injection of 1 per cent. formalin or 20.0 to 40.0 mls. of Lugol's iodine ; Pals (1960) used a number of chemotherapeutic agents. It does not at present appear that there is a specific cure for besnoitiosis.

Other methods of control have not yet been investigated under field conditions.

CHAPTER XIV

CHEMOTHERAPY

THE EVALUATION OF DRUGS : THE PRINCIPAL DRUGS USED TO CONTROL PROTOZOA

CHEMOTHERAPY is the branch of therapeutics that deals with the treatment of parasitic infections with drugs. The term should be reserved for treatment which involves the actual destruction or suppression of parasites. Symptomatic treatment of disease, without a direct effect on the parasite, should not be included in a study of chemotherapy.

THE HISTORY OF CHEMOTHERAPY

Up till the end of the nineteenth century the treatment of disease was largely empirical ; the major advances associated with the work of Ehrlich could not take place until advances in pathology had established the nature of parasitic disease in animals. One of the earliest uses of a chemical substance for the control of a parasitic disease was in the control of nagana in Africa, where arsenic proved to have some effect. Later Ehrlich tested the effects of a series of dyes which showed a differential affinity for the parasite as compared with the tissues of the host. Although this early work was not entirely satisfactory from the practical point of view, it did demonstrate conclusively that it was possible to damage a parasite without destroying the host. Ehrlich's original conception of the "lock and key" mechanism of chemotherapeutic action, *i.e.* the idea that successful chemotherapy was dependent upon finding a chemical substance of a molecular structure which would be "accepted" by the parasite, has been modified in the light of more recent thought, to include the similar effect of chemical substances on the metabolic processes of the parasite. As stated by Findlay (1950) "The conception that chemotherapeutic agents compete with cell metabolites for enzyme systems essential to the metabolism of the cell is a logical restatement in terms of enzyme chemistry of Ehrlich's original conception of cell receptors."

THE EVALUATION OF CHEMOTHERAPEUTIC SUBSTANCES

The evaluation of a chemotherapeutic substance resolves itself into

- (a) An assessment of its parasitocidal activity.
- (b) The determination of the best means of administration.
- (c) An assessment of the effects of the drug on the host.
- (d) Establishment of the best methods of use under field conditions.

The assessment of parasitocidal activity

In the earliest stages of an investigation a drug is usually included in a "screening" operation wherein a number of related compounds are submitted to a biological test which has been standardised for the particular parasite in a particular host. The criterion of efficacy may be fixed as the rate of mortality, red blood cell count, actual parasitæmia and so on, according to experience of the type of disease the parasite is known to cause under laboratory conditions. With an increase in our limited knowledge of the way in which parasites are affected by chemotherapeutic substances it becomes increasingly possible for industrial chemists to set out to synthesise compounds of a particular structure which is likely to have a particular therapeutic activity. New drugs are often structurally related to older ones.

The determination of the best means of administration

The evaluation of a drug for use against a protozoon parasite may be particularly difficult when the parasite has a life-history which includes various stages of development. It may be found, as with *Eimeria tenella*, that there is an optimum level of a sulphonamide above which there is an interference with the earlier developmental stage, on the completion of which effective therapy depends. It may then be necessary to recommend an interrupted type of treatment which allows the essential early development to occur. Under experimental conditions in the laboratory it is necessary to determine whether the drug is more effective when given in the food or in the water or by intravenous or intra-peritoneal injection, or by some other route.

The assessment of the effects of the drug on the host

Because the fundamental structure and the basic metabolic processes of all living organisms are similar it is reasonable to suppose that what is poisonous to the parasite is likely to be poisonous to the host. Toxicity to the host may be evident at the time of treatment (as with overdoses of Quinuronium sulphate, for example) or some time after treatment (*e.g.* the photosensitivity associated with dimidium bromide). In the laboratory it is necessary to test a new drug at a range of concentrations that allows for the almost inevitable misuse under practical conditions. A drug with a narrow margin between therapeutic and toxic doses should not be used unless no substitute can be found. In practice, a true assessment of probable toxicity often cannot be obtained until the drug has been tested under field conditions. Often, predisposing factors may be responsible for the occurrence of hitherto unsuspected toxicity ; drugs may be toxic to the animal when in some particular stage of development, *e.g.* young or old, or they may be toxic only when some particular diet is given. Under some commercial conditions, *e.g.* in a Broiler House for the production of table poultry, toxicity which causes a slight temporary check in the rate of growth may mean the exclusion of a particular drug which might be allowed for growing pullets.

The best methods of use under field conditions

In general, veterinary chemotherapy differs from its human counterpart in that the price of a drug is likely to be a most important factor in assessing its practical worth. Ease of administration is another point of vital importance, particularly when considering the treatment of large numbers of animals in under-developed countries. It is, as a rule, much better for a drug to be administered subcutaneously than intravenously, which is technically more difficult and certainly more time-consuming. It is quite useless to suggest for poultry a drug which requires repeated oral dosing. Drugs which are given in the drinking water must be stable on dilution and must remain in solution under conditions of varying *pH*. Above all, the effects of a drug must be consistent from batch to batch.

SPECIAL TERMS USED IN CHEMOTHERAPY

The therapeutic index of a drug

The therapeutic index, or chemotherapeutic index (C.I.) of a drug is indicated by

$$\text{C.I.} = \frac{\text{Maximum tolerated dose}}{\text{Minimum curative dose}}$$

It is obviously essential that the difference between the dose of a drug which is lethal to the parasite (minimum curative dose) and the largest amount that is tolerated by the host (maximum tolerated dose) should be sufficiently big to permit its use as a remedy without serious danger to the patient. The greater the value of the therapeutic index, therefore, the safer the drug is in use. It must be remembered, however, that the index may vary considerably for the same parasite in different species of host or when administered by different routes even in the same host. Accurate comparisons of toxicity can be made on the 50 per cent. point of response. One means of expressing the therapeutic index is, accordingly, the ratio between the median lethal dose (L.D. 50, *i.e.* the dose that kills 50 per cent. of the animals) and the median effective dose (C.D. 50, *i.e.* the dose that cures 50 per cent. of the animals suffering from the effects of the parasite). Thus

$$\text{C.I.} = \frac{\text{L.D. 50}}{\text{C.D. 50}}$$

This may not, however, directly indicate the real curative dose or the real toxic dose. A better comparison may be the L.D. 0.1 (the dose that kills all animals except 0.1 per cent.) and the C.D. 99.9 (the dose that cures all animals except 0.1 per cent.).

The drug equivalents of compounds

The drug equivalent of a compound is assessed as the dose necessary to produce the same response (usually the L.D. 50) as a unit dose of some other compound of known therapeutic efficacy. Thus, the efficacy of a series of coccidiostatic drugs might be assessed in terms of sulphadimidine, a compound of known and high potency.

Synergism and potentiation

There is some difference of opinion as to the correct nomenclature to adopt when describing the different ways in which chemotherapeutic substances may act together.

Many people accept *Synergism* as being a generic term indicating that drugs act together on a parasite. Synergism includes a particular type of co-activity called *Potentiation*. This is considered to occur when the effect of the simultaneous use of drugs is greater than would have been anticipated from observation of their effects when used alone.

Potentiation may occur in a variety of ways. With the sulphonamides and pyrimethamine it is believed that there is separate and sequential action on the same metabolic pathway which leads to the synthesis within the parasite of the nucleoproteins which are essential to the development of the schizonts.

Alternatively, potentiation may occur because one substance assists the penetration or absorption of another. It can be shown that while a normal strain of *Trypanosoma brucei* suspended in a solution of *Suramin* may fail to absorb drug, a strain which has previously been treated with trypaflavine gradually increases in permeability to *Suramin*.

Drug resistance (drug-fastness)

The occurrence, among parasites ordinarily susceptible to the action of drugs, of strains which are no longer susceptible, introduces one of the most important problems of chemotherapy. Drug-fastness was originally noted in Ehrlich's laboratory soon after work on trypanosomiasis had commenced. A short time afterwards it was found that *T. gambiense* in the field became resistant to *Atoxyl* (sodium p-amino-phenylarsonate). Nowadays, there are few if any compounds, used for the treatment of trypanosomiasis, against which drug-fast strains have not arisen. It is believed that drug-fast strains arise because of the selective effect of treatment on field populations of the parasite which consist of mixed strains varying in their susceptibility to the drug. The effect of treatment is then merely to weed out those strains which are more than ordinarily susceptible, while allowing the increase of the more resistant strains. This process of selection is particularly marked with some parasites, notably *Trypanosoma*

congolense which seems to be remarkably genetically labile, fresh strains of the parasite constantly arising within any population.

It is apparent that such a process of selection will be assisted by the use of drugs at concentrations less than those which would be lethal to most if not all of the population. Under experimental conditions resistant strains can arise as the result of giving a drug in quantity sufficient to remove parasites temporarily from the blood while not eliminating them. The relapse strains, if similarly treated, gradually become fast. In the field, resistance is therefore commonly associated with under-dosing, or with treating a parasite for a limited period of time with a drug which is merely suppressive.

Cross-resistance can be shown, *i.e.* a parasite which becomes fast to one drug may become fast to another ; not necessarily a closely related compound. In some instances, the establishment of a strain which is fast to one type of drug may indirectly induce fastness to another. Thus a strain of trypanosome which is fast to tartar emetic (ordinarily very rare) may be induced by first producing an atoxyl-fast strain. Two or three moderate doses of tartar emetic will then produce fastness. The actual mechanism of drug resistance is not understood but the phenomenon appears usually to be associated with the development of an ability on the part of the parasite to absorb less of the drug. Thus it can be shown that trypanosomes resistant to dyes remain unstained by these dyes ; trypanosomes resistant to arsenic absorb less arsenic than usual and parasites resistant to the aromatic compounds fail to absorb these drugs. With the aromatic arsenicals, drug resistance develops rather easily and is related more to the resistance to the substituted phenyl radical than to the arsenic itself.

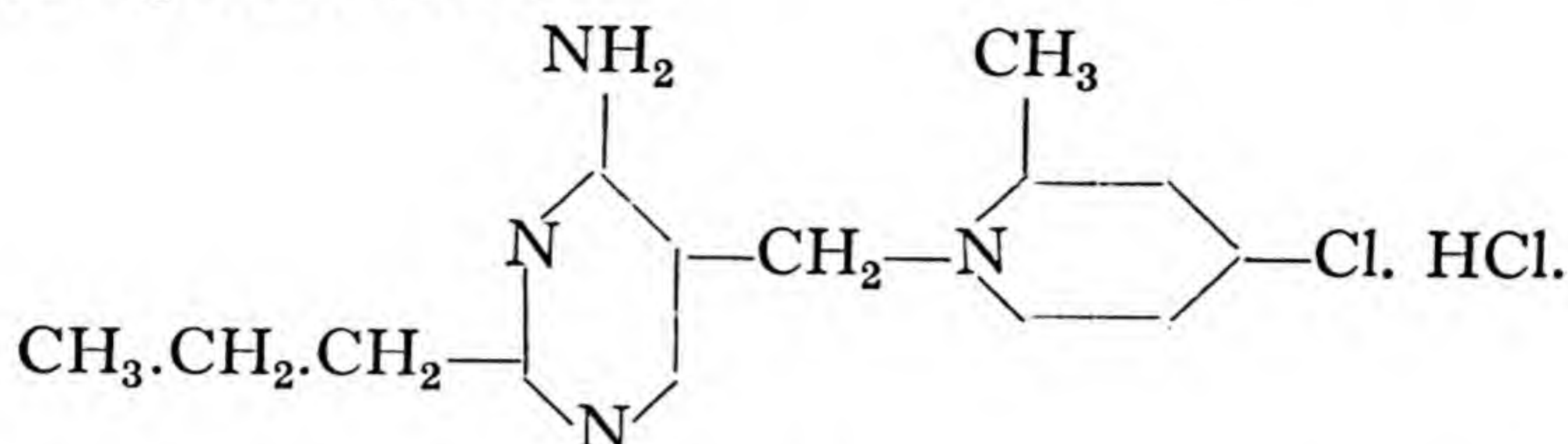
Chemotherapeutic interference

This is a phenomenon which, like drug resistance, is associated with a decreased capacity on the part of the parasite to absorb drug. It is apparently initiated by previous contact with another drug. Thus the injection, a few hours previously, of paraflavine will interfere with the action of such drugs as trypaflavine or tartar emetic.

THE PRINCIPAL DRUGS WHICH HAVE PROVED ACTIVE AGAINST PROTOZOA

Amprolium

1-(4-amino-2-*n*-propyl-5-pyrimidinyl-methyl)-2-picolinium chloride hydrochloride.



Uses. Prevention of cæcal and intestinal coccidiosis in chickens and turkeys.

Dose. Usually 0.0125 per cent. of the food fed continuously. Cuckler, Cobb, McManus and Ott (1961) have reported its efficacy against the coccidia of turkeys at 0.003 per cent. to 0.025 per cent. of the food.

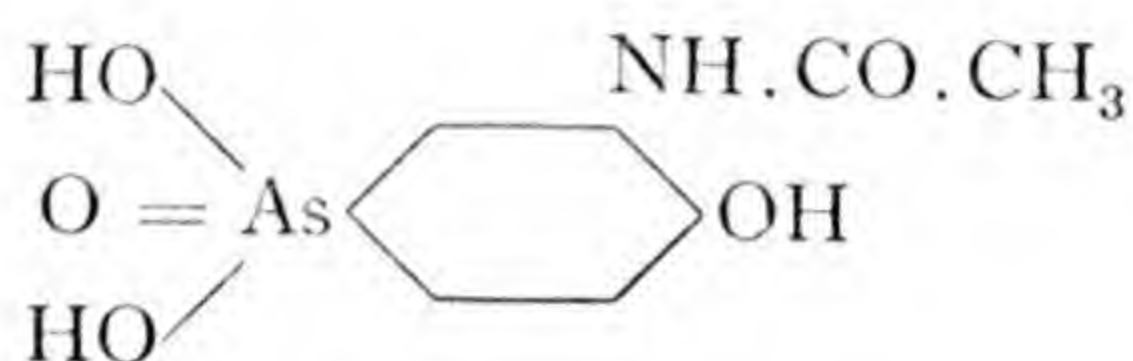
Arsenic

Arsenic had been used for the treatment of malaria for some centuries B.C. but it had been administered almost exclusively by inunction till A.D. 1200, when arsenious oxide began to be prescribed internally for malaria. This preparation was administered orally by Balfour and Livingstone for the treatment of sleeping sickness and in 1893 Linguard showed that it was valuable in the treatment of surra. It was not, however, until the less toxic organic preparations were introduced and found to have a specific value in the treatment of syphilis that the real possibilities of arsenical compounds were appreciated. Pentavalent and trivalent compounds are in use. Because the arsenic acids are relatively inactive in the destruction of trypanosomes *in vitro* but active *in vivo*, whereas the arsenoxides are active *in vitro* it was deduced that the body tissues must have the power of reducing the pentavalent arsenic acids to the trivalent arsenoxides which then destroy the trypanosomes. Active arsenicals seem to be concentrated very rapidly in the body of the trypanosome, where the arsenic combines with the cellular elements thus permitting the diffusion into the parasite of more drug. The actual

way in which the arsenic attacks the trypanosome is still not clear, but it is probably through the inactivation of essential -SH groups. The carbohydrate requirements of most trypanosomes are very great and it is possible that arsenic affects the respiration of the parasite in some way.

Pentavalent compounds

Acetarsol (*stovarsol*, *spirocid*, 3-acetamido-4-hydroxyphenyl-
arsonic acid)

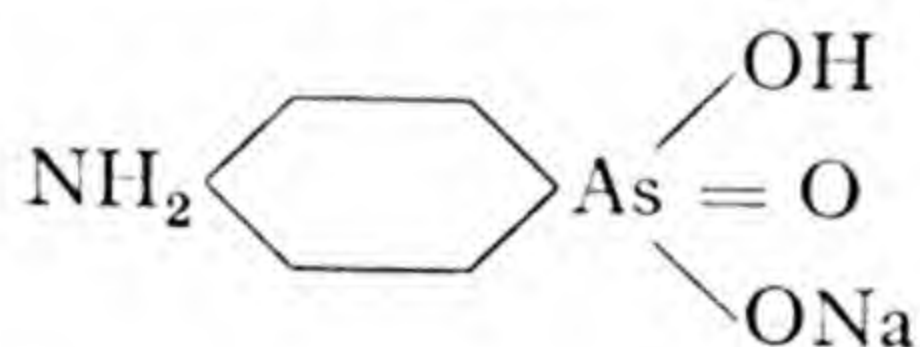


Uses. In addition to being a useful tonic and of value in various skin diseases, acetarsol has some effect in the prevention of histomoniasis in turkeys. A weak action against amœbic dysentery has been reported and the drug has been used in the treatment of canine babesiasis.

Dose. For the prevention of histomoniasis in turkeys sodium acetarsol is added to the drinking water at a concentration of 0.01 per cent.

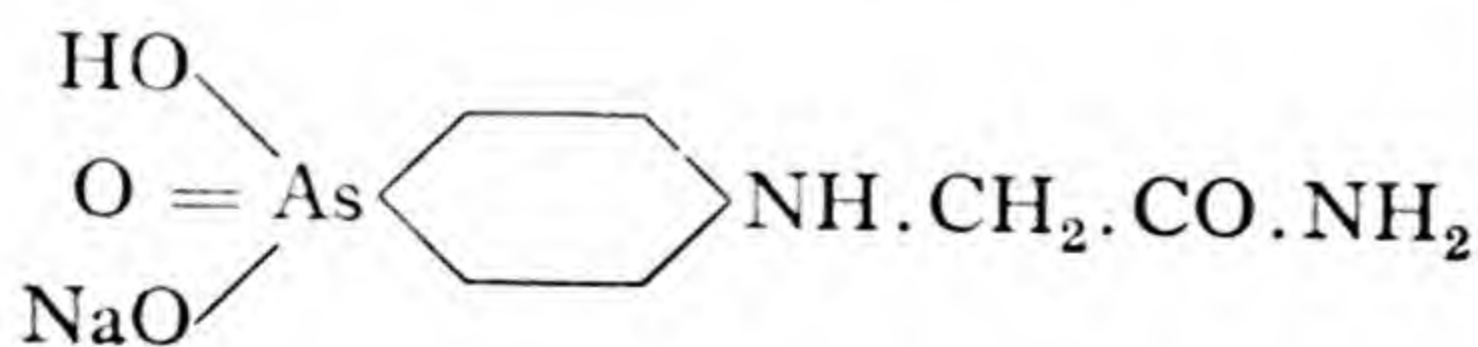
Atoxyl (Soamin ; sodium *p*-aminophenylarsonate).

Sodium aminophenylarsonate is a white crystalline powder, soluble in water, with a saline taste.



Uses. Atoxyl is active against trypanosomes of the *T. brucei* group but is liable to induce toxicity, evinced by atrophy of the optic nerve. It has been largely superseded by other drugs.

Tryparsamide (Sodium *p*-carbamylmethylanilphenyl-
arsonate.)



This substance controls infection with *T. brucei* and *T. evansi* in laboratory animals, but has not proved so successful in field practice. It has been used in human medicine for the treatment of *T. gambiense* in cases where the cerebro-spinal fluid has become involved.

Trivalent arsenical compounds

Butarsen

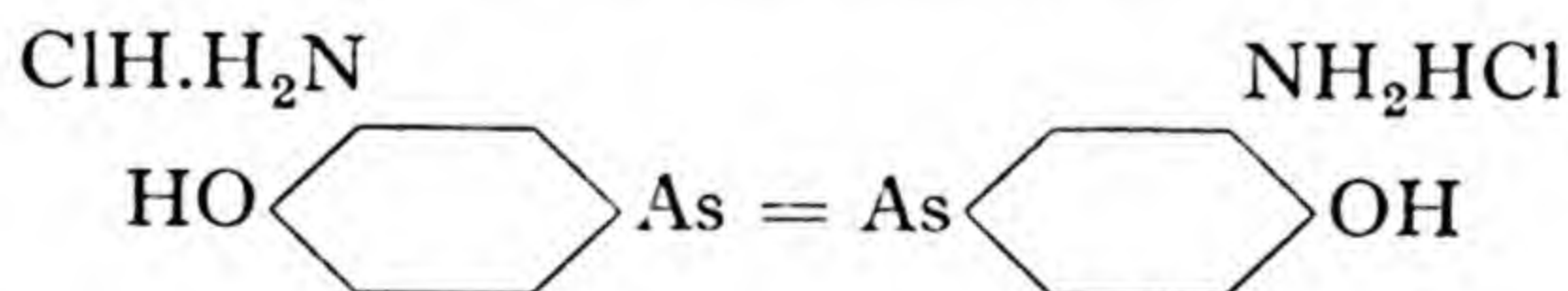
4-(*p*-oxarsinophenyl) butyric acid is a white compound insoluble in water but dissolving in alkali to form the highly soluble sodium salt.



Uses. In human trypanosomiasis the drug has been shown to have a rapid effect on the parasites in the peripheral blood stream and in the cervical lymph nodes. It is quite successful in early cases of *T. gambiense* but is probably not effective if the C.N.S. is involved. It may be used for the treatment of cases which are resistant to other arsenicals. It has been used for the treatment of *T. evansi* and *T. equiperdum* in horses, and for *T. evansi* in dogs.

Dose. For the horse—six injections every other day at the rate of 1.25 mg. per kilo. For dogs 3.5 mg. to 5.0 mg. per kilo has been partially successful.

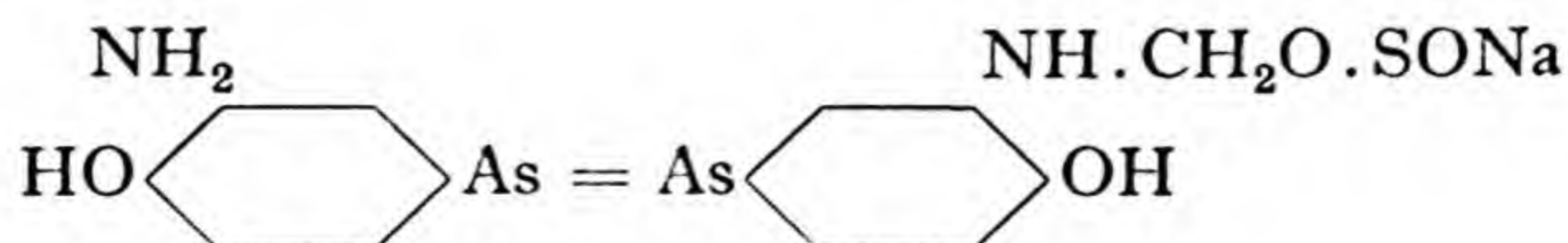
Arsphenamine (Salvarsan, arsenobenzol)



This substance has never been used to any great extent in veterinary medicine. Neoarsphenamine has replaced it.

Neoarsphenamine (Neosalvarsan, Novarsenobenzol, Neokhar-sivan, Novarsenobillon).

This is a yellowish powder, soluble in water with a neutral reaction.

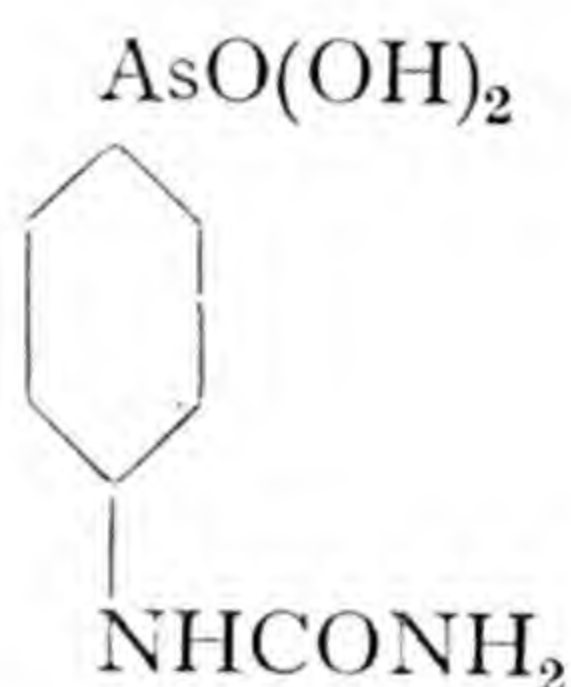


Solutions of this substance are unstable and must be freshly prepared.

Uses. Neoarsphenamine has been recommended for use against histomoniasis, bovine anaplasmosis, bartonellosis and *Babesia gibsoni* infections in dogs, against *Eperythrozoon* in sheep and in laboratory mice.

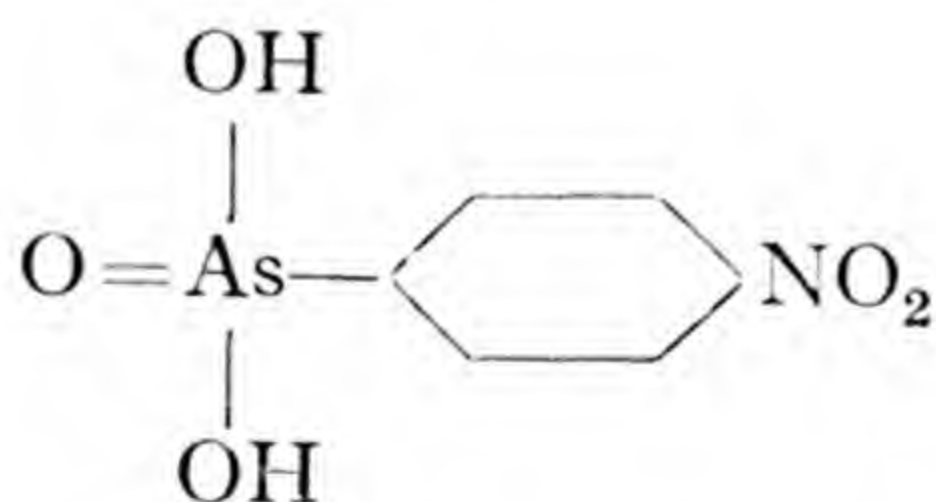
Dose. 45 mg./kg. for *E. ovis* in sheep and 15 mg./kg. for *Bartonella* in the dog, given intravenously.

4-carbamylaminophenylarsonic acid (p-ureidobenzenearsonic acid "Carbarsone", "Histocarb").



This drug is used for the control of amœbiasis, balantidiosis and *Trichomonas vaginalis* infection. It is available also for the control of histomoniasis in poultry at a recommended dose of 0.0375 per cent. of the food.

4-nitrophenylarsonic acid (4-nitrobenzenearsonic acid—"Histostat").



Uses. For the control of *Histomonas*, the drug is reported to be very effective when fed in the food at a concentration of 0.0125 per cent. to 0.075 per cent. or when given in the drinking water at 0.006 per cent. to 0.04 per cent. as a preventive.

Toxicity. When used continuously at 0.026 per cent. of the drinking water there is a decrease in egg production and growth rates are affected.

Arsenic-antimony compounds

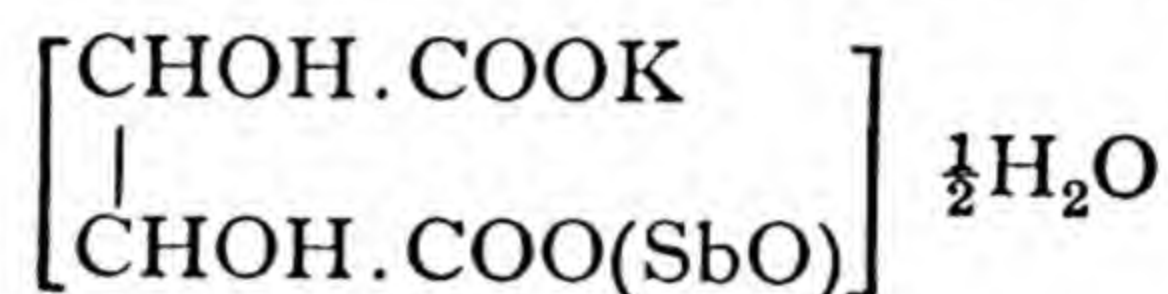
Sdt-386-B

The composition of this drug has not been divulged but it is said to contain 18 per cent. of arsenic and 20 per cent. of antimony. It has been claimed that it is a specific for oraya fever (human bartonellosis) when given intravenously in doses of 0.1 to 0.3 gm. Neitz (1940) reported that it was a specific for eperythrozoonosis in sheep.

Compounds of antimony

Tartar emetic (antimony potassium tartrate)

The structural formula is usually represented as :—



Physical characteristics. Colourless transparent crystals, soluble in water to form a slightly acid solution.

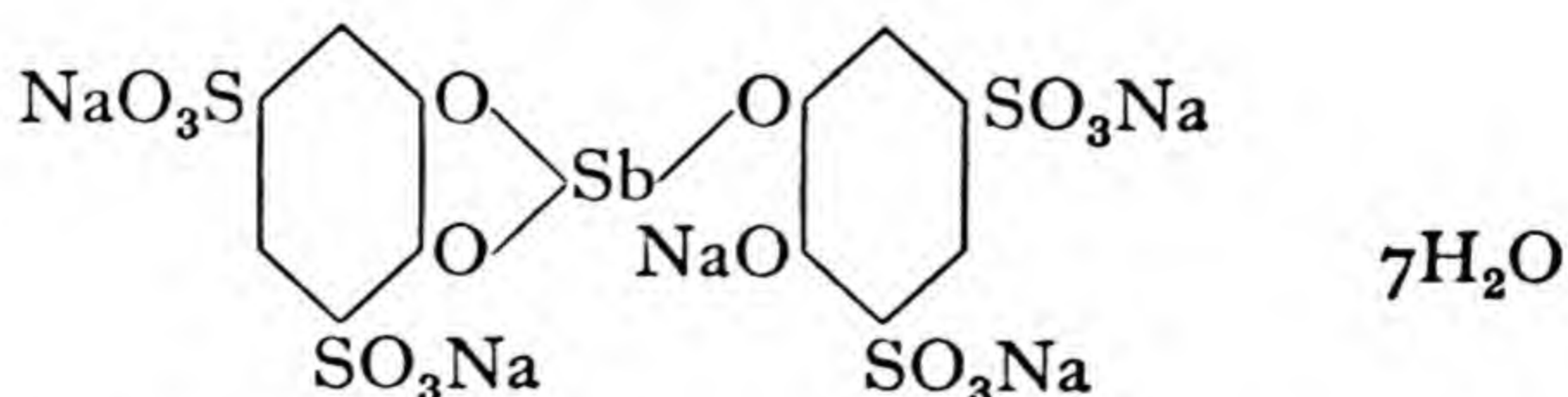
Uses. Tartar emetic is used in the treatment of leishmaniasis and for *T. evansi*, *T. congolense* and *T. vivax*.

Dose. For cattle 1 gm. weekly given intravenously for four to five weeks. Solutions must be freshly prepared and care taken to avoid putting drug outside the vein.

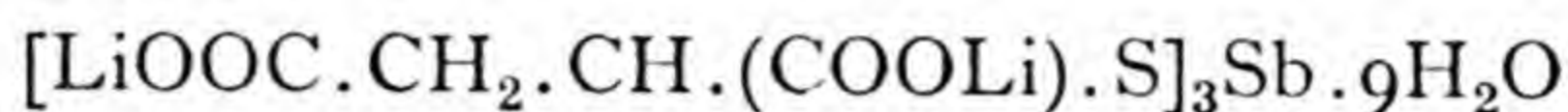
Antimosan

This substance is very similar in its action to tartar emetic but is less toxic and can be injected subcutaneously or intramuscularly. It has been used for the treatment of leishmaniasis and *T. congolense* infections, particularly in the dog. In composition it does not differ essentially from neo-antimosan (Stibophen).

Stibophen (neo-antimosan, fouadin)



Stibophen is similar in its action to antimosan and has been used in particular against *T. congolense* infection in cattle. Four to five injections, at weekly intervals, are necessary.

Anthiomaline

This compound of antimony and lithium has an effect on *T. evansi* and has been recommended for the treatment of canine leishmaniasis.

Bismuth compounds

Preparations of bismuth were used in very early times in the treatment of alimentary inflammation and in 1889, Balzar suggested the use of bismuth in the treatment of syphilis.

Bismuth salicylate

A suspension of this compound called *Todorit* has been used as an intramuscular injection for the treatment of bovine piroplasmosis caused by *B. bovis* and *B. argentina*.

Silver compounds

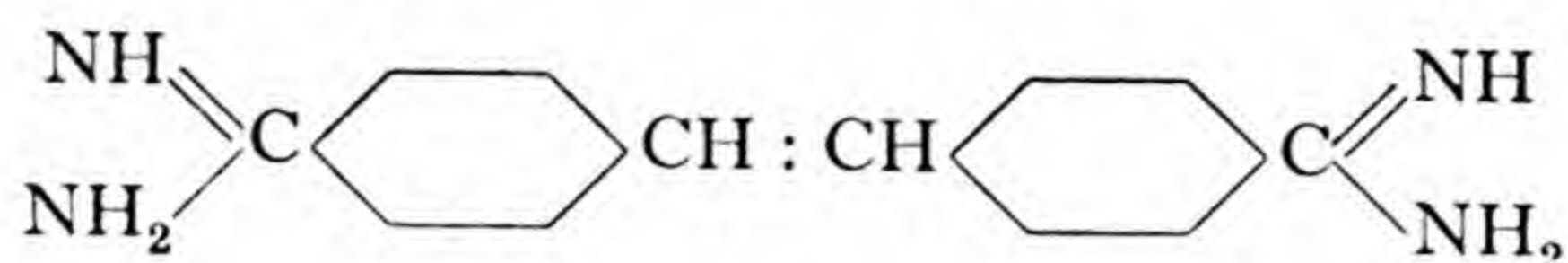
Silver compounds are not used to any considerable extent in veterinary medicine.

Ichthargan

This silver preparation is given intravenously as a 1 per cent. solution at the rate of 1.5 gm. for a 200-400 kg. beast for the treatment of piroplasmosis. Some action on *B. bovis*, *B. bigemina*, *B. berbera* and *B. major* has been reported. Two or three injections may be necessary.

The amidines

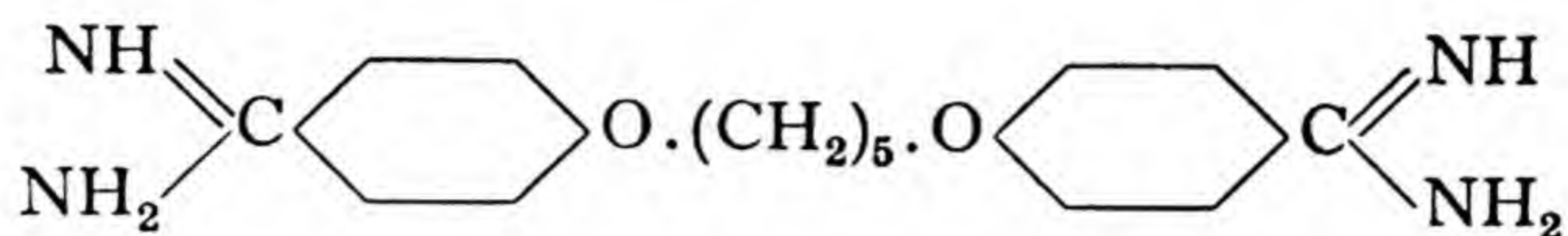
Lourie and Yorke (1939) examined the trypanocidal activity of a number of aromatic diamidines and found that some were trypanocidal both *in vitro* and in small laboratory animals. Four have found use in human or in veterinary medicine.

Stilbamidine (4 : 4'-diamidinostilbene)

This compound has not so far been used extensively in veterinary medicine although it has some effect against *T. congolense* in

cattle. For *B. canis* in dogs 1.5 mg./kg. is given subcutaneously as a 1.0 per cent. to 1.5 per cent. solution. Adler and Tchernomoretz (1940) used the drug against *Babesia ovis* and *B. bigemina*.

Pentamidine (Lomidine 4 : 4'-diamidino 1 : 5-diphenoxypentane)

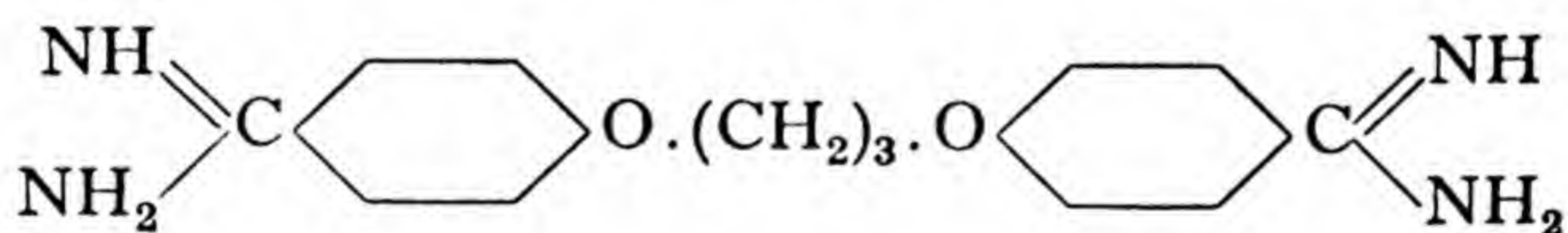


Pentamidine has been used extensively in the prophylaxis of human trypanosomiasis due to *T. gambiense* in Central and West Africa and is effective also against kala-azar. It has recently been introduced into veterinary medicine for the treatment of *Babesia bovis*.

Dose. For cattle :—one or two subcutaneous or intramuscular injections of a 10 per cent. aqueous solution containing 1 gm. of the salt (diisethionate).

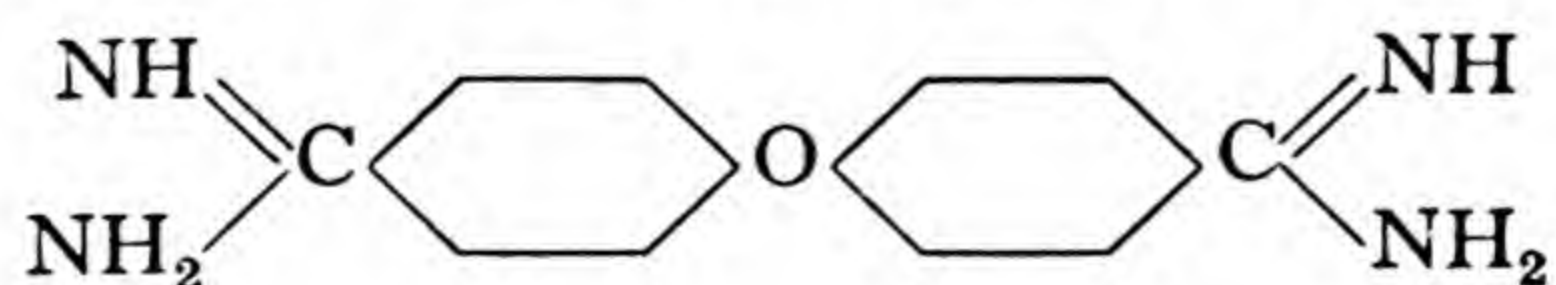
Toxicity. In the treatment of trypanosomiasis Daubney and Hudson (1941) have reported delayed toxicity. 10 mg./kilo may be immediately toxic.

Propamidine (4 : 4'-diamidino 1 : 3-diphenoxypropane)



Uses. This drug has not found extensive use in veterinary medicine. It has been used as a single dose of 5 mg./kg. or on two successive days at a dose of 2.5 mg./kg. against *Babesia canis*. Use of the drug has been reported by Carmichael and Fiennes (1941). It is said to keep indefinitely as a 1 per cent. solution.

Phenamidine (4 : 4'-diamidino diphenyl ether)



Dose (by subcutaneous injection)

Cattle and horses. 600 mg./50 kilos body weight, as a 40 per cent. aqueous solution.

Dogs. 10 mg./kg. as a 5 per cent. solution. A single dose is usually effective but may be repeated on the next day.

Toxicity. Occasional immediate toxicity of an anaphylactic type may be encountered. Usually the animal recovers. All the diamidines tend to cause a transient fall in blood pressure. This can be partially avoided by the (pre-injection) use of intravenous calcium gluconate.

Berenil

4 : 4'-diamidinodiazobenzene aceturate (Fussgänger, 1955).



Uses. (a) Against trypanosomes :—relatively poor effect against the *brucei* group ; better against *congolense* (Davey, 1957). A poor action against *T. simiae* (Bauer, 1955). Used against *T. brucei* in the horse but there may be a local reaction. Leach (1961) found 3.5 mg./kg. ineffective against some strains of *T. evansi* in camels. High blood concentrations are of relatively short duration *i.e.* the drug is primarily curative rather than prophylactic ; particularly useful against strains of trypanosomes resistant to other drugs.

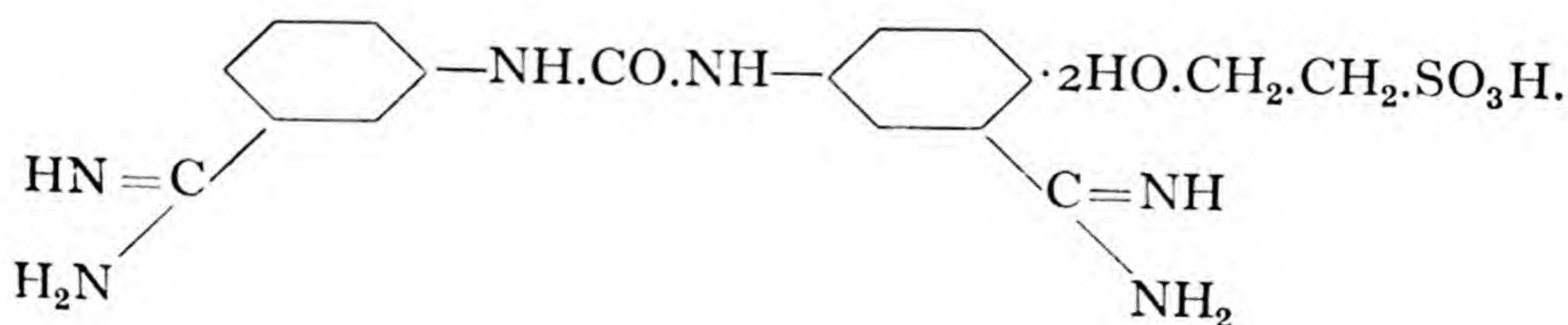
(b) Against *Babesia* (see Section on *Babesia*).

Dose. 2.0 to 3.5 mg./kg. usually by deep i/m. injection.

Toxicity. In some instances 7 mg./kg. has been given safely but Leach found this toxic for adult camels (Leach, 1961).

Diampron

3 : 3'-Diamidinocarbanilide di-isethionate (May and Baker 5062-A ; Amicarbalide).



Physical characters. A white crystalline solid, melting at about 202°C . It dissolves readily in water up to a concentration of nearly 100 per cent. w./v. at 25°C . The pH of a 10 per cent. solution is about 6.0.

Uses. Ashley, Berg and Lucas (1960) and Lucas (1960) reported that 3 : 3 diamidinocarbanilide di-isethionate (May and Baker, 5062A, Diampron) was effective against *B. rodhaini* in white mice and *B. divergens* in splenectomised calves at dosage levels of 5-40 mg./kg. and the value against *B. divergens* was confirmed in a field trial by Beveridge *et al.* (1960).

Shone *et al.* (1961) showed an effect against *B. bigemina* at 10 mg./kg. Kemron *et al.* (1960) tested the drug against *Babesiella* (*Babesia*) *berbera* in cattle and found that 10 mg./kg. intramuscularly was effective when administered at the onset of illness. Treatment at a later stage was much less effective or ineffective.

Dose. 5 mg./kg. given intramuscularly or subcutaneously (with "Diampron" this is equivalent to 0.5 ml. of a 50 per cent. w./v. aqueous solution for each 50 kg.).

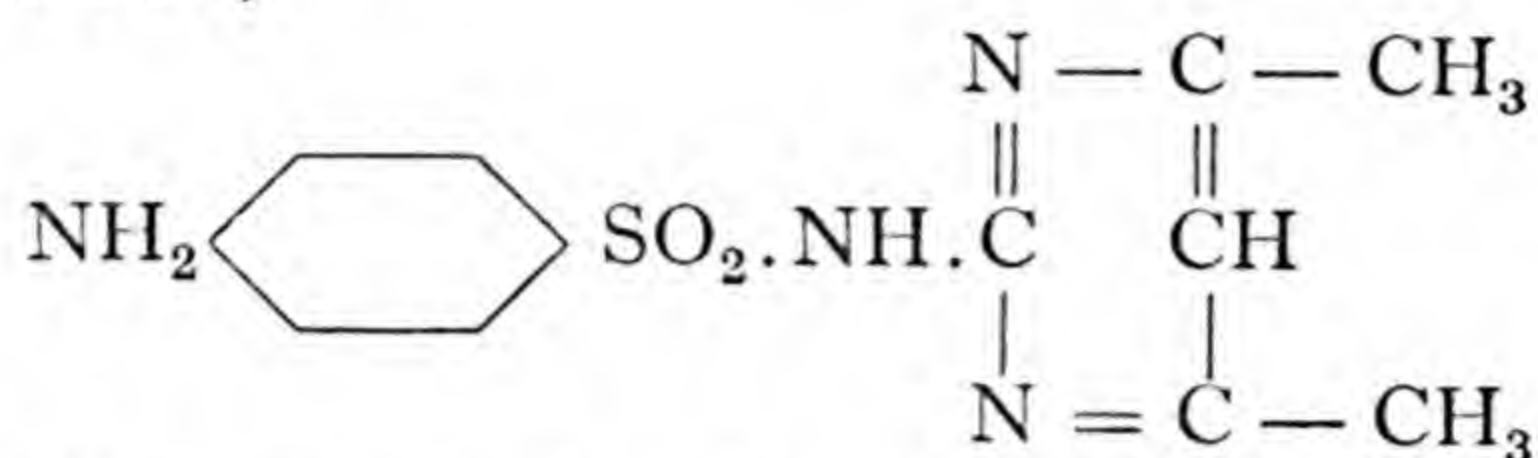
Treatment may be repeated and with peracute disease the dose may be increased to 10 mg./kg.

Toxicity. Deep intramuscular injection is safer than the subcutaneous route (Shone *et al.*, 1961). The maximum tolerated dose for calves is more than 40 mg./kg. but at this level there is some transient toxicity which may be observed under field conditions even at 10 mg./kg.

The sulphonamides

The sulphonamides are active against a wide range of organisms. *p*-aminobenzoic acid is an essential factor for the growth of these parasites. If a sulphonamide, which contains a nucleus like *p*-aminobenzoic acid, is present in excess, the parasite attempts to utilise the sulphonamide instead of *p*-aminobenzoic acid and the appropriate enzyme system in the parasite becomes blocked. Sodium salts of the sulphonamides are much more soluble than the parent substances and are often used preferentially. Sulphanilamide itself, *p*-aminobenzenesulphonamide, has limited action against *Eimeria*.

Sulphadimidine (Sulphamezathine, Sulphamethazine, sulphadimethylpyrimidine, 4:6-dimethyl-2-sulphanilamido-pyrimidine)



Physical characters. A white powder, almost odourless and tasteless. Sparingly soluble in water. The sodium salt is soluble in water and remains in solution as long as it remains strongly alkaline. Dilute solutions deteriorate after about 12 hours.

Uses. For the treatment of coccidiosis caused by various species of *Eimeria*.

Dose

Poultry. Sulphadimidine in the food at 0.4 per cent. for a maximum of six days, preferably in interrupted treatment or the sodium salt in the drinking water at 0.2 per cent.

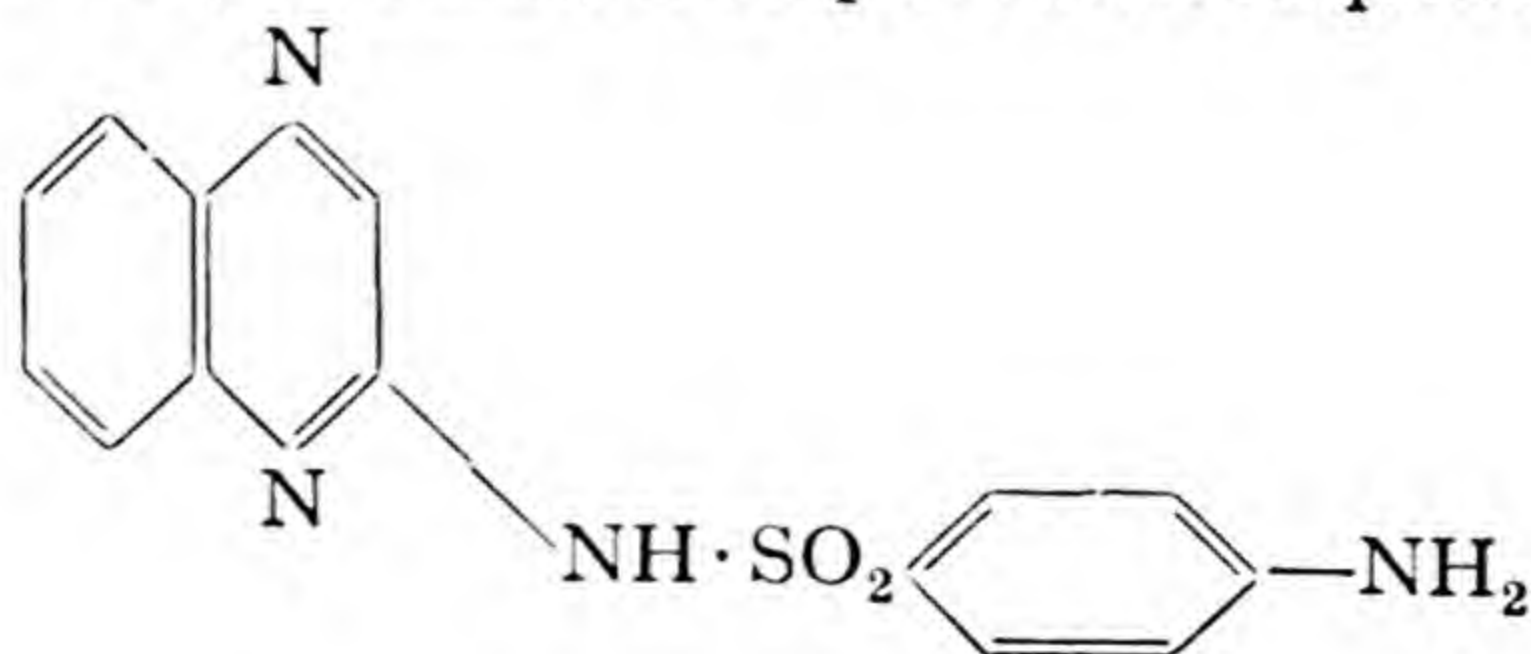
For intestinal coccidiosis an initial three days at 0.2 per cent. should be followed after two days without treatment by two further three-day periods of treatment, interrupted by two days without treatment. The two latter treatment periods should be at the reduced concentration of 0.1 per cent.

Cattle, sheep and goats. 0.2 gm./kilo initial dose followed by half this dose for five days.

Rabbits. 0.5 gm./day for an average sized rabbit.

Toxicity. Sulphadimidine is usually safe in use. Mammals should be encouraged to drink freely when under treatment. With poultry there is risk of poisoning if more than the recommended concentrations are given, particularly with birds four to ten weeks of age.

Sulphaquinoxaline (Embazin, 2-sulphanilamidoquinoxaline)



Physical characters. A yellow powder, like sulphadimidine sparingly soluble in water but dissolving readily in alkaline solution to form the sodium salt. Very dilute solutions do not remain stable for more than about 12 hours.

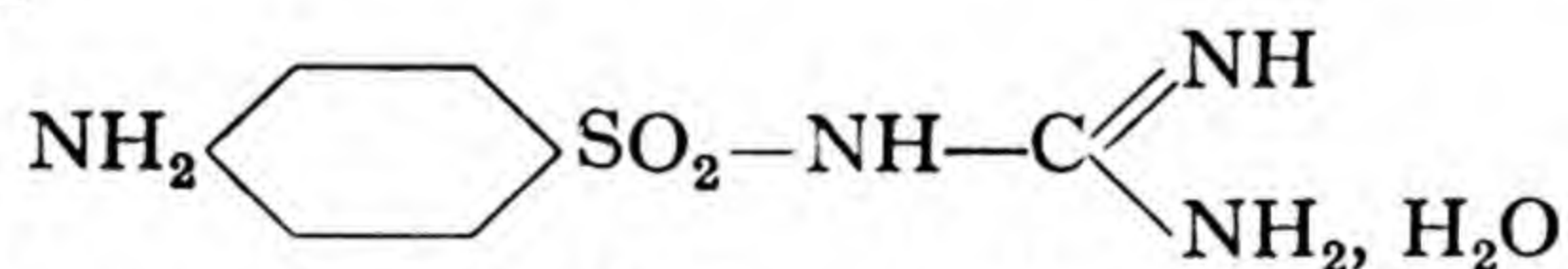
Uses. For the control of coccidiosis, particularly in turkeys.

Dose. For poultry and rabbits, 0.05 per cent. of the sodium salt in the drinking water for a maximum of six days preferably in an interrupted type of treatment.

For so-called "preventive treatment" the drug has been used at a concentration of about 0.01 to 0.02 per cent. (of sulphadoxine) in the food. For cattle an initial dose of 0.15 gm./kilo followed by half this dose for four or five days has been used for curative treatment.

Toxicity. In chickens, use of the drug has been associated with a toxic syndrome characterised by the presence of hæmorrhagic or necrotic foci in the spleen and liver and by hæmorrhages into the body cavities and muscle tissues. This condition is included in the syndrome of mixed ætiology known in America as the "hæmorrhagic syndrome".

Sulphaguanidine (*p*-aminobenzenesulphonylguanidine monohydrate)



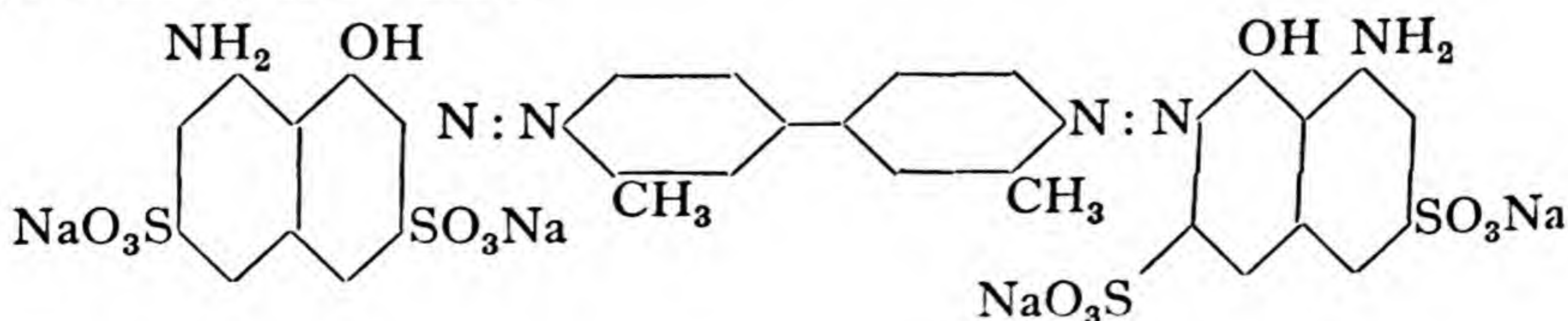
Physical characteristics. White needle-like crystals, darkening in light, tasteless. Solubility 1 in 1000 water.

Uses. The slow and incomplete absorption of sulphaguanidine has led to its virtual replacement in the treatment of coccidiosis by the more soluble sulphonamides.

Naphthalene derivatives

Trypan blue

This is the sodium salt of ditolyl diazo-bis-8-amino-1-naphthol 3 : 6-disulphonic acid.



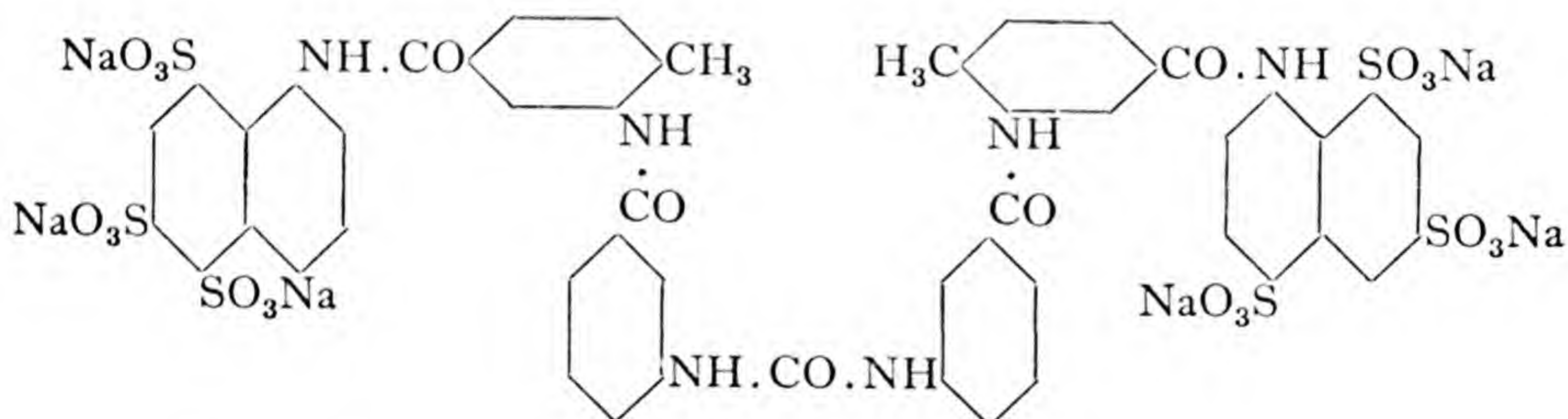
Uses. Trypan blue is effective against the large species of *Babesia*—*B. canis*, *B. bigemina*, *B. motasi* ; Not against *B. berbera* or *B. major*.

Dose. For *B. bigemina* in cattle 1 gm./100 kg. intravenously. For *B. canis* 4 to 5 ml. of a 1 per cent. solution intravenously.

Toxicity. Abscess formation and sloughing follows injection outside the vein. Tissues are stained blue-green for several months after the injection. Occasional toxicity is observed but is usually associated with overdosing. There is difficulty in breathing, acute œdema of the lungs, fever and muscular tremors.

Antrypol

Description. Antrypol is usually marketed in the anhydrous form. Trade names for the same product include—Bayer 205 ; Fourneau 309 ; Germanin ; Naganol ; Moranyl ; Belganyl ; Suramin ; Naphuride Sodium.



Physical characters. This is a white or cream-white soft microcrystalline powder, readily soluble in cold water. The anhydrous form can be sterilised by heat. It is very hygroscopic and should be kept in closed containers.

Uses. Antrypol is used as a 10 per cent. solution in sterile water or saline. It is effective against both *Trypanosoma gambiense* and *T. rhodesiense* in man. In domestic animals, *T. evansi* and *T. brucei* and other members of their groups are sensitive. *T. equinum* and *T. equiperdum* are somewhat less sensitive. (It is fairly easy to cure horses in the early stages of disease but the dose is dangerously high.) *T. vivax* and *T. congolense* are resistant. Antrypol is, however, often used in combination with other trypanocidal drugs.

Dose. (a) By intravenous injection for curative treatment

Horse and donkey—0.35 to 0.5 gm./50 kg.

Cattle and buffalo—0.6 gm./50 kg.

Camels—3.0 to 5.0 gm./camel depending on the size.

Dogs and pigs—0.007 gm./kg.

(b) For prophylaxis—by subcutaneous injection

Horses—1 gm. every three months.

Camels—4 gm. every two months.

The prophylactic use of Antrypol is particularly useful in horses. The drug is retained for long periods in the plasma and tissue protein. It can be detected in the plasma for as long as five months after injection.

Toxicity. Toxicity is particularly evident in horses. Symptoms, which may follow use of the drug at the minimum levels at which it is therapeutically active, may include albuminuria, urticaria, swellings under the skin of the legs, belly, throat, etc., eczematous eruptions of the mouth and anus, and laminitis. There is really no margin between the toxic and the therapeutic dose and safe effective therapy depends on the experience of the veterinary surgeon. Calcium lactate intravenously is reported to help avoid toxicity. All animals other than the horse appear to be much more tolerant.

Drug resistance. Resistance has been reported with *T. evansi* in the camel, but is not usually a serious hazard.

Suramin complexes

Suramin is acid and can form complexes with a number of basic substances. Williamson and Desowitz (1956) and Williamson (1957) made a number of complexes using trypanocidal drugs. The chemical and physical nature of the complexes tends to limit their absorption and diffusibility when they are injected and hence permits the possibility of "depot" therapy against cattle trypanosomiasis *i.e.* they can be injected to form depots of drug which are released very slowly over a long period of time and hence afford considerable periods of protection. Ethidium bromide-suramin complex was one of the most promising, giving up to 13 months' protection with an apparent reduction in toxicity of the complexed drugs. Thus antrycide seemed to be tolerated at eight times the usual maximum dose. More recent work has,

however, tended to show that although the complexing reduced the systemic toxicity of the drugs there was a tendency for a strong local reaction with the formation of plaques of necrotic tissue. These might burst, with consequent severe systemic reaction, sometimes the death of the animal and with at the best loss of the depot of drug and hence loss of protection from the trypanosome. Stephen and Williamson (1958 and 1961) have made efforts to avoid this complication but with little apparent success.

Quinoline derivatives

The discovery of the activity of these compounds followed from the study of the chemical composition of quinine, an alkaloid isolated from cinchona bark, which was used by natives of South America for the treatment of malaria.

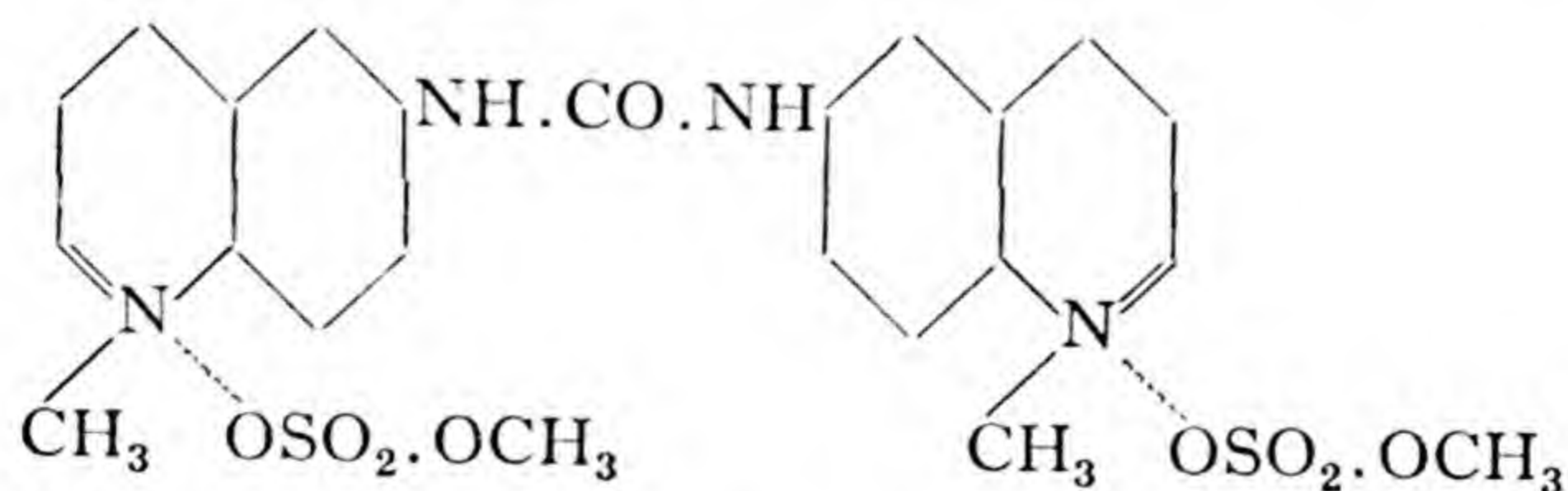
Quinine itself, Plasmoquin (Plasmochin), Yatren (Loretin, Chiniofon Quinoxyl), Vioform (Enterovioform) Ambisyl, Enterochin, Quinambicide and Drodquin are all in use in human medicine for the treatment of malaria or of amœbic dysentery.

Surfen C (Congasin) (bis(2-methyl-4-amino-6-quinolyl melamine))

The variation in composition of different samples of this drug contra-indicated its use in the field. It is active against *T. brucei* and *T. congolense*.

Quinuronium sulphate

This is 6 : 6'-di(*N*-methylquinolyl) urea dimethosulphate. It is marketed by different companies under the following trade names :—Acapron, Pirevan, Babesan, Piroparv, Zothelone, Piroplasmin and Acaprin.



Physical characters. This is a stable, bright yellow crystalline powder, the melting point being 240° C. It is usually sold in aqueous solution, which should be protected from the light.

The solution is not self-sterilising, but can be sterilised by heating with a suitable bactericide at 100° C.

Uses. Good effects have been reported against nearly all species of *Babesia* but *B. gibsoni* appears to be resistant. The effect on *Nuttallia equi* and against *B. ovis* is not so marked as on *B. bigemina* or *B. canis*.

Dose. (Acaprin). The drug is given subcutaneously. Intravenous injection is contra-indicated. For large animals a 5 per cent. solution is used :—

Horses—0·6 ml./50 kg.

Cattle—1·0 ml./50 kg.

Large pigs—1·0 ml./50 kg.

For small animals a 0·5 per cent. solution is used :—

Sheep—2·0 ml./10 kg.

Dogs—0·25 ml./5 kg.

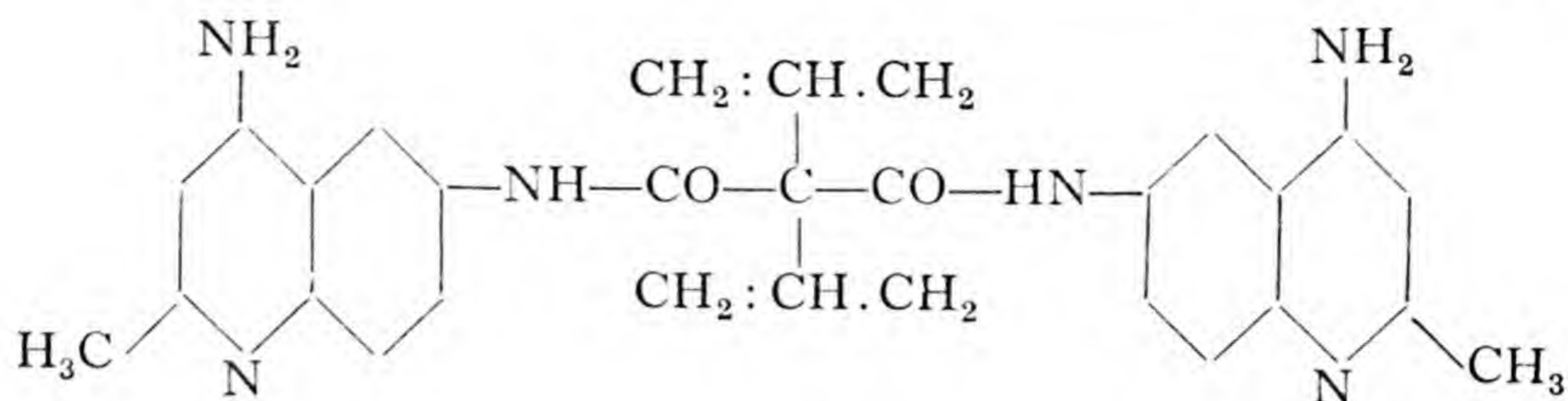
Small pigs—0·5 ml./5 kg.

Toxicity. Quinuronium sulphate has a specific action on the parasympathetic system and there may be alarming reactions following treatment. These include salivation, vasodilatation (with sweating), copious urination, diarrhoea, panting and a fall in blood pressure sometimes with collapse and death.

After intravenous injection toxicity may be evinced within a few minutes: after subcutaneous injection in 20-30 minutes. Apart from this parasympathetic effect there is no particular toxicity, although it has been suggested that intoxication may follow the massive accumulation of dead parasites in the blood stream following treatment. To avoid the possibility of toxicity the total dose may be given in two or three parts at intervals of a few hours. Circulatory stimulants such as adrenaline (with calcium gluconate) can be given as an antidote to collapse. There is no evidence that repeated doses at the therapeutic level are dangerous but a second dose should be given within 24 hours of the first in order to avoid sensitivity.

Bayer 7602

This drug was introduced specifically for the treatment of *T. cruzi* infection in man.

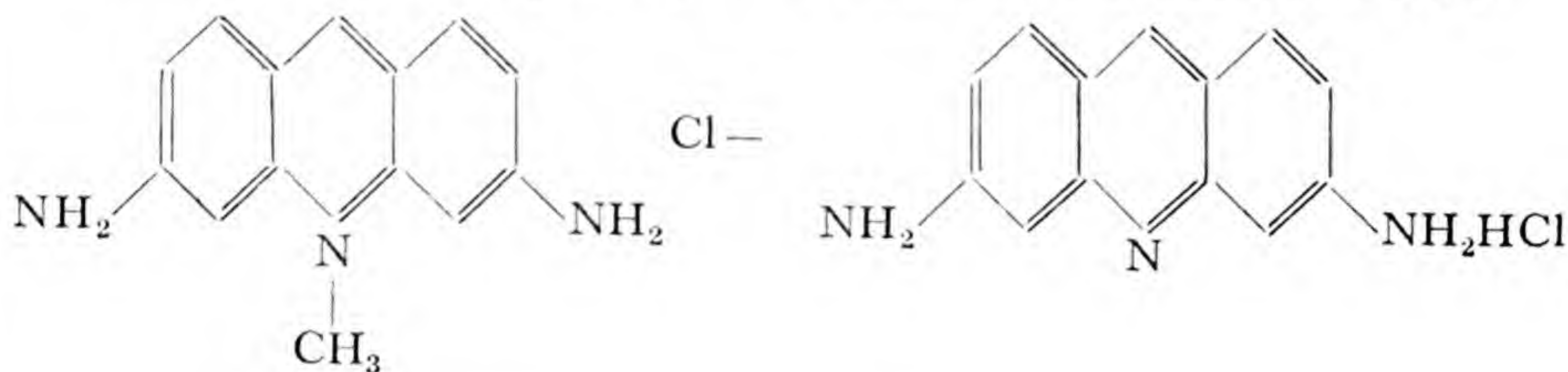


Uses. In human medicine it is given by intramuscular injection at the rate of 7-12 mg./kg. given at intervals of five to seven days.

Acridine derivatives

Acriflavin (Trypaflavin, Gonacrine, Flavin, Euflavin)

This is a mixture of 2 : 8-diamino-10-methylacridinium chloride and a small quantity of 2 : 8-diamino acridinium chloride.

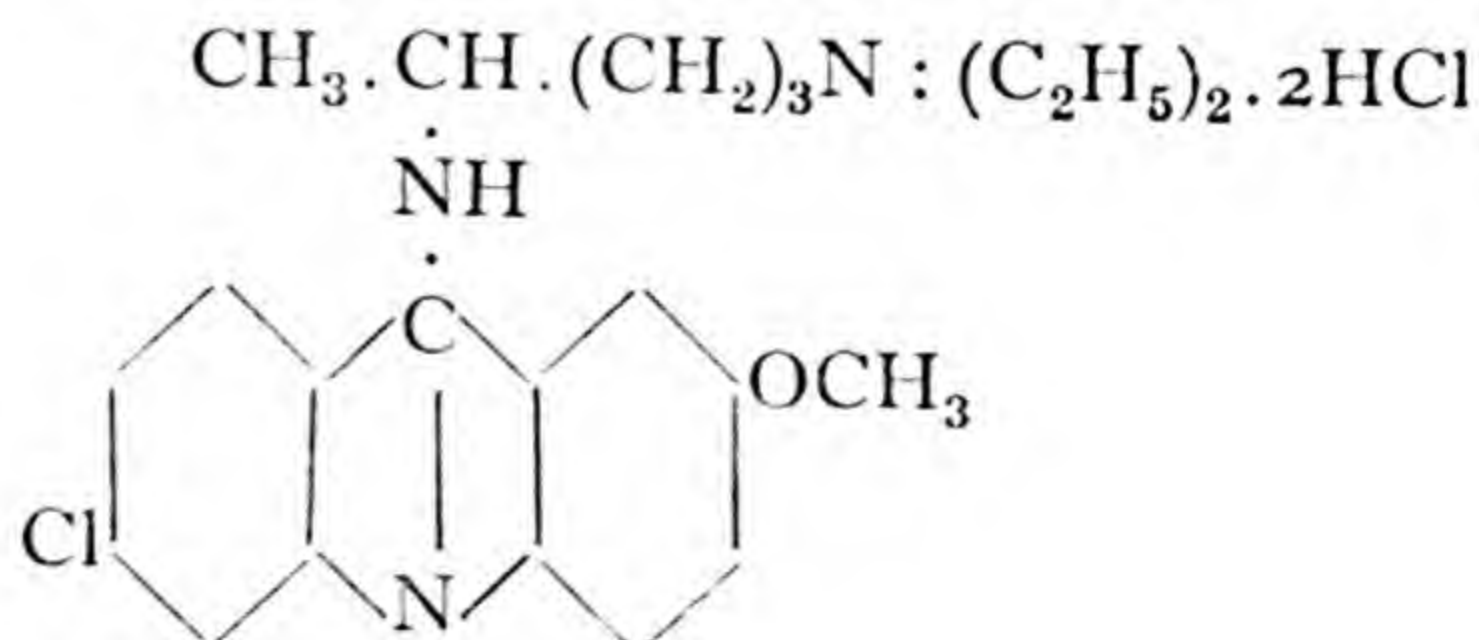


Physical characters. An orange-red crystalline powder with an acid taste and no odour ; soluble one in three of water.

Uses. This product has been recommended for use against *B. bigemina*, and *B. bovis*, *B. caballi* and *B. equi* in horses, *B. ovis* in sheep and *B. canis* in dogs.

Dose. For *B. bigemina* in cattle 100 to 200 ml. of a 1 per cent. solution given intravenously. For *B. berbera* the recommended treatment is up to four doses of 0.5 gm. at intervals of 24 hours.

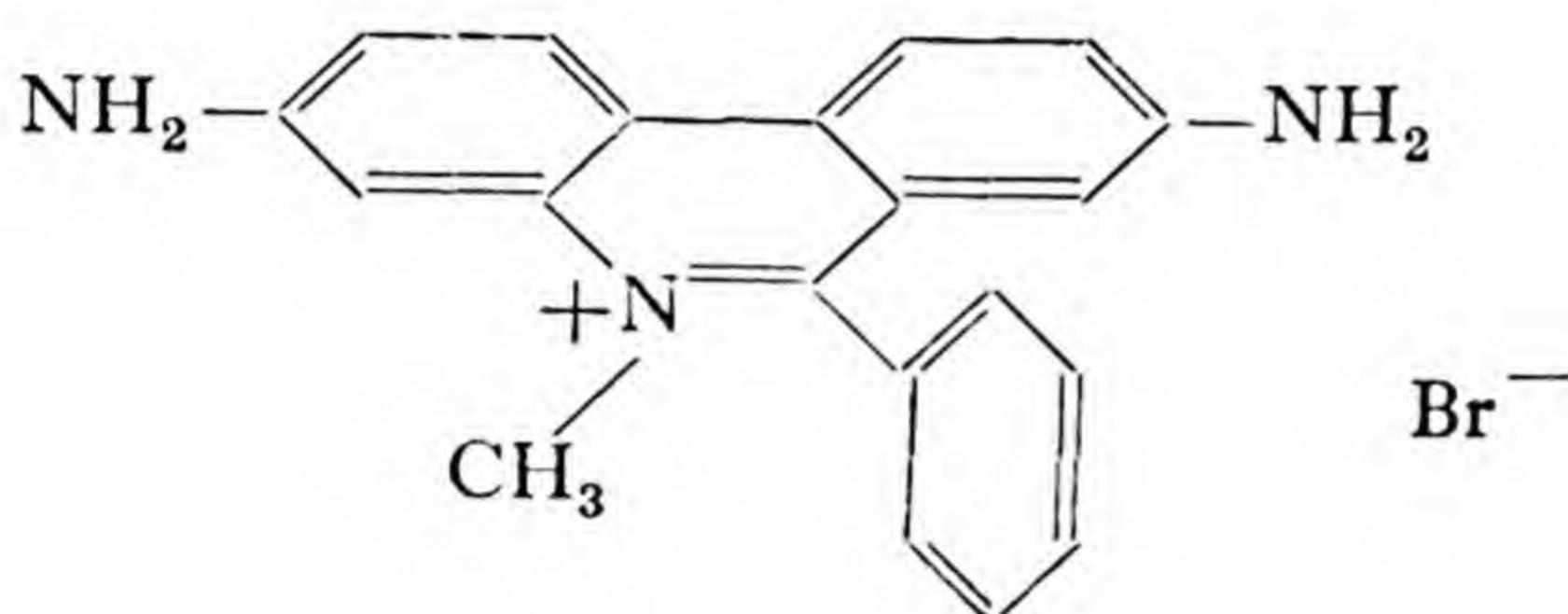
Mepacrine (Atebrin, Quinacrine, Mecryl)



Uses. Mepacrine has a specific action against human malaria and is sometimes used in veterinary medicine for the control of bovine coccidiosis. The recommended dose (Mecryl) for cattle is 1 gm./100 kilos, dissolved in a litre of water and given as a drench. The dose is repeated for four or five days.

Phenanthridinium compounds

Dimidium bromide (2 : 7-diamino-9-phenyl-10-methylphenanthridinium bromide)



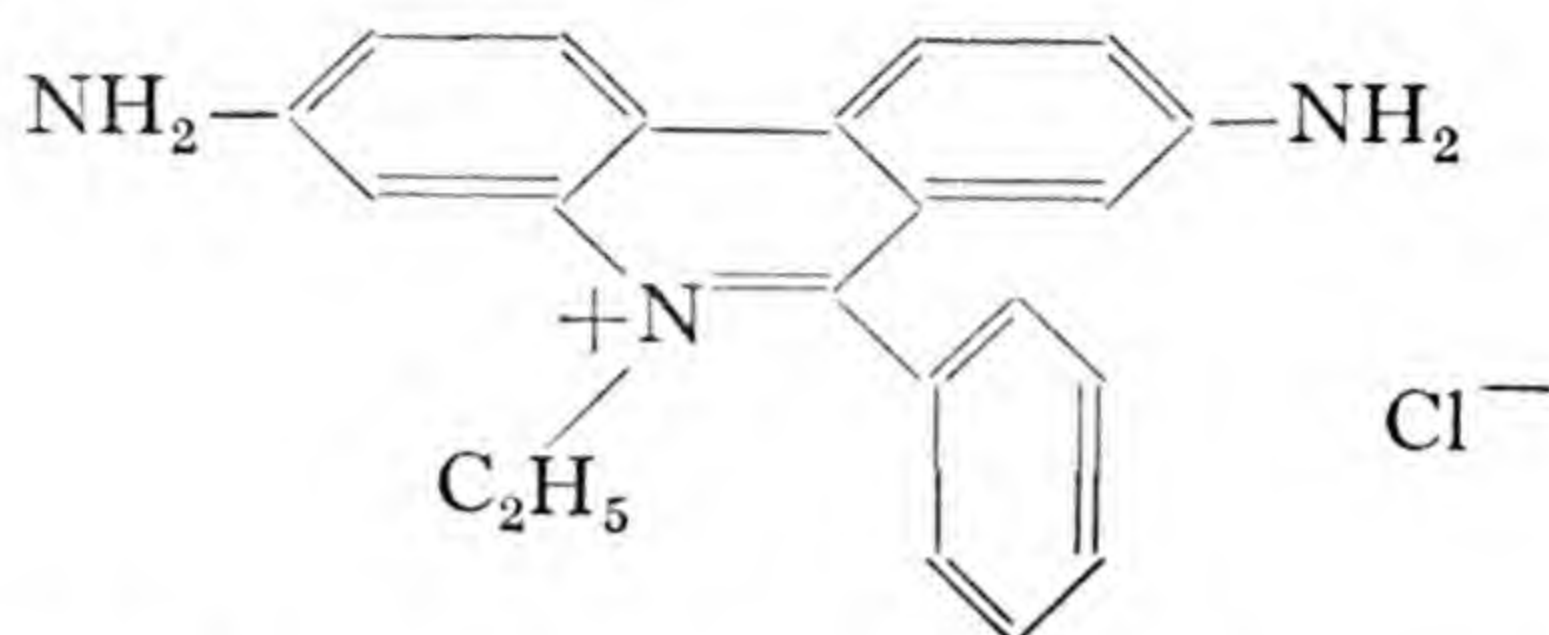
Physical characters. A deep-red crystalline powder, freely soluble in water.

Uses. Active against *Trypanosoma congolense* and *T. vivax*. It is not effective against *T. brucei* or *T. evansi*.

Dose. 1 to 2 mg./kg. live weight given as a sterile 1-2 per cent. aqueous solution intravenously or subcutaneously. One or two doses are usually successful. Some recent evidence suggests that intramuscular injection is preferable.

Toxicity. Irritation at the site of injection: often photosensitisation particularly at doses of 4 mg./kg. or more. At 6 mg./kg. there may be salivation, shallow breathing, muscular incoordination, coma and death. The ratio between the toxic and the curative dose is not very great; 3 mg./kg. may cause transient liver damage, reaction at the site of injection and the delayed toxicity syndrome. In some such instances there is anorexia on about the thirty-fifth day after treatment and the cow loses condition rapidly. Post-mortem examination shows that the mucous membranes are icteric, the liver is pale and yellow in patches, hard to touch and with the cut surface yellow and greasy. The gall bladder is greatly enlarged and the kidneys finely mottled and congested.

Novidium chloride ("Novidium"; Homidium chloride; Ethidium chloride; 2:7-diamino-9-phenyl-10-ethylphenanthridinium chloride)



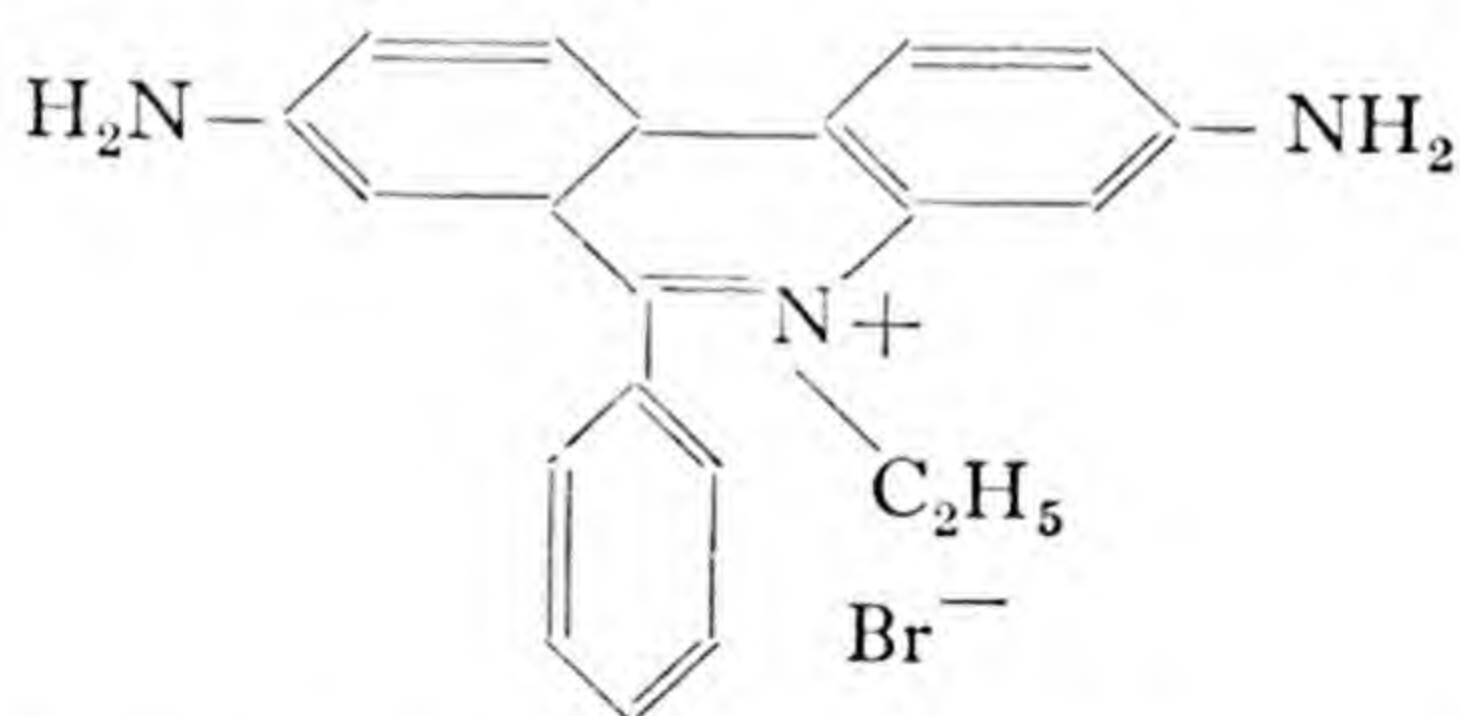
Uses. Stated to be very effective against *T. congolense* and *T. vivax*.

Dose. Given by deep intramuscular injection as a 2.5 per cent. w/v aqueous solution at the rate of 1 mg./kg. body weight.

Toxicity. Transient local reactions. Photosensitisation has not so far been reported.

Ethidium bromide (Homidium bromide; 2:7-diamino-9-phenyl-10-ethylphenanthridinium bromide)

This is a homologue of Dimidium bromide, the methyl group having been replaced by ethyl.



Physical characters. Ethidium bromide crystallises as dark-red elongated plates, melting at 248-249° C. with decomposition. It dissolves readily in water to give a 3.5 per cent. solution which withstands boiling.

Uses. Effective against *T. congolense* in cattle and against early infection with *T. vivax*. The drug has been shown to be effective against strains of trypanosomes resistant to dimidium bromide and Antrycide. Up to six weeks' prophylaxis against *T. congolense* and *T. vivax* has been shown under field conditions.

Dose. In cattle, subcutaneous injection of a 1 per cent. solution at the rate of 0.1 mg. to 0.3 mg. per kilo body weight

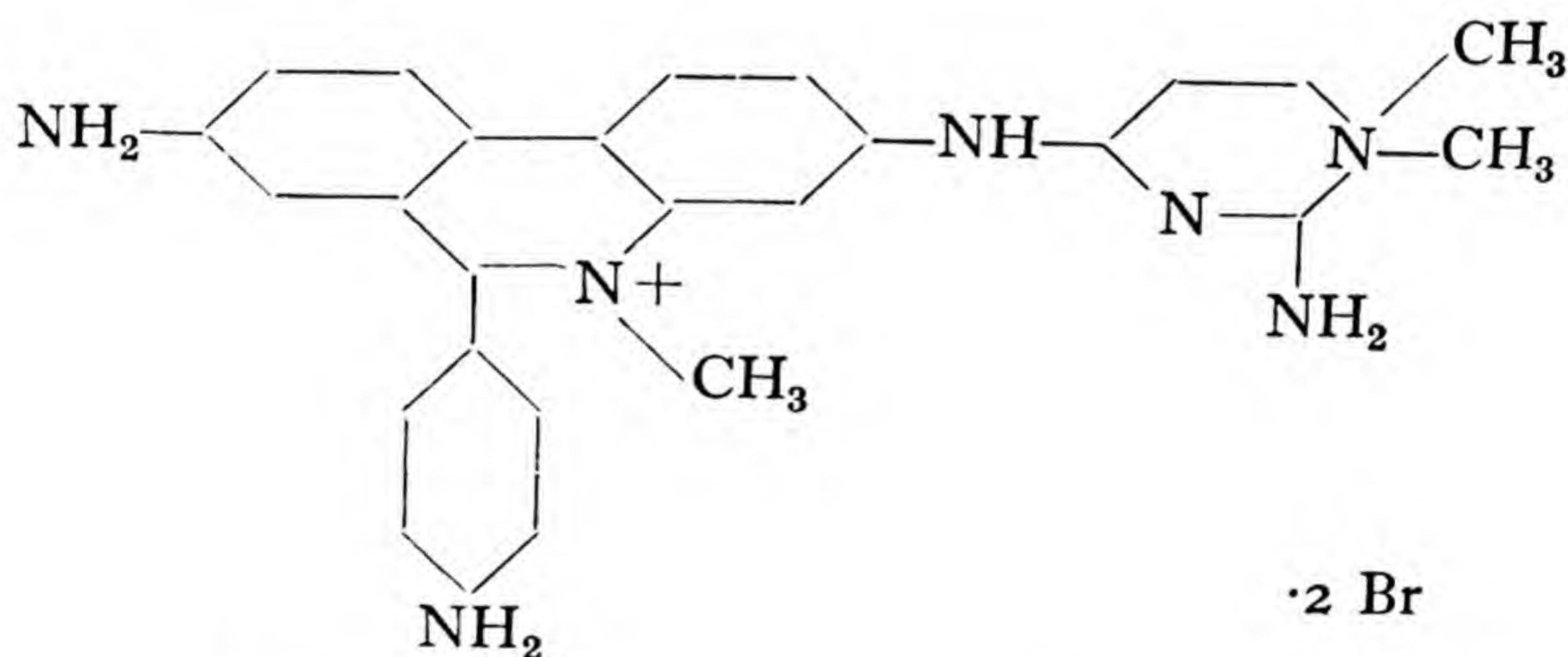
will usually control *T. congolense*. Higher doses are recommended for *T. vivax* and if 1.0 mg./kg. or more is used intramuscular injection produces less reaction.

Toxicity. Treatment with 10 mg./kg. may be followed by death or transient liver damage. As a rule up to 5 mg./kg. is tolerated by cattle. There is a local reaction with œdematous swellings and thickening of the subcutaneous tissues which may leave small scars.

Drug resistance. Resistance to ethidium bromide can occur and such resistant strains cannot be treated with dimidium bromide.

Prothidium

(2-amino-7-(2'-amino-1' : 6'-dimethylpyrimidyl-4'-amino-9-p-aminophenyl-10 methyl phenanthridinium dibromide). (Watkins and Woolfe, 1956).



A series of different salts has been issued for trial : the dibromide (Prothidium) is usually used as a 4 per cent. solution given subcutaneously.

Uses. Against *T. congolense*, Robson and Milne (1957) found that 2 mg./kg. protected cattle for up to six months : Smith (1959) used a 2 per cent. suspension in distilled water to protect W. African short-horn zebu cattle which were exposed to high densities of *Glossina pallidipes*. Several authors agree that 2 mg./kg. or 4 mg./kg. prothidium bromide as a 4 per cent. solution, given subcutaneously, gives from 70 to as much as 300 days' protection against trypanosomiasis (Smith 1959 ; Leach and el Karib, 1960 ; Lyttle, 1960 ; Robson, 1961).

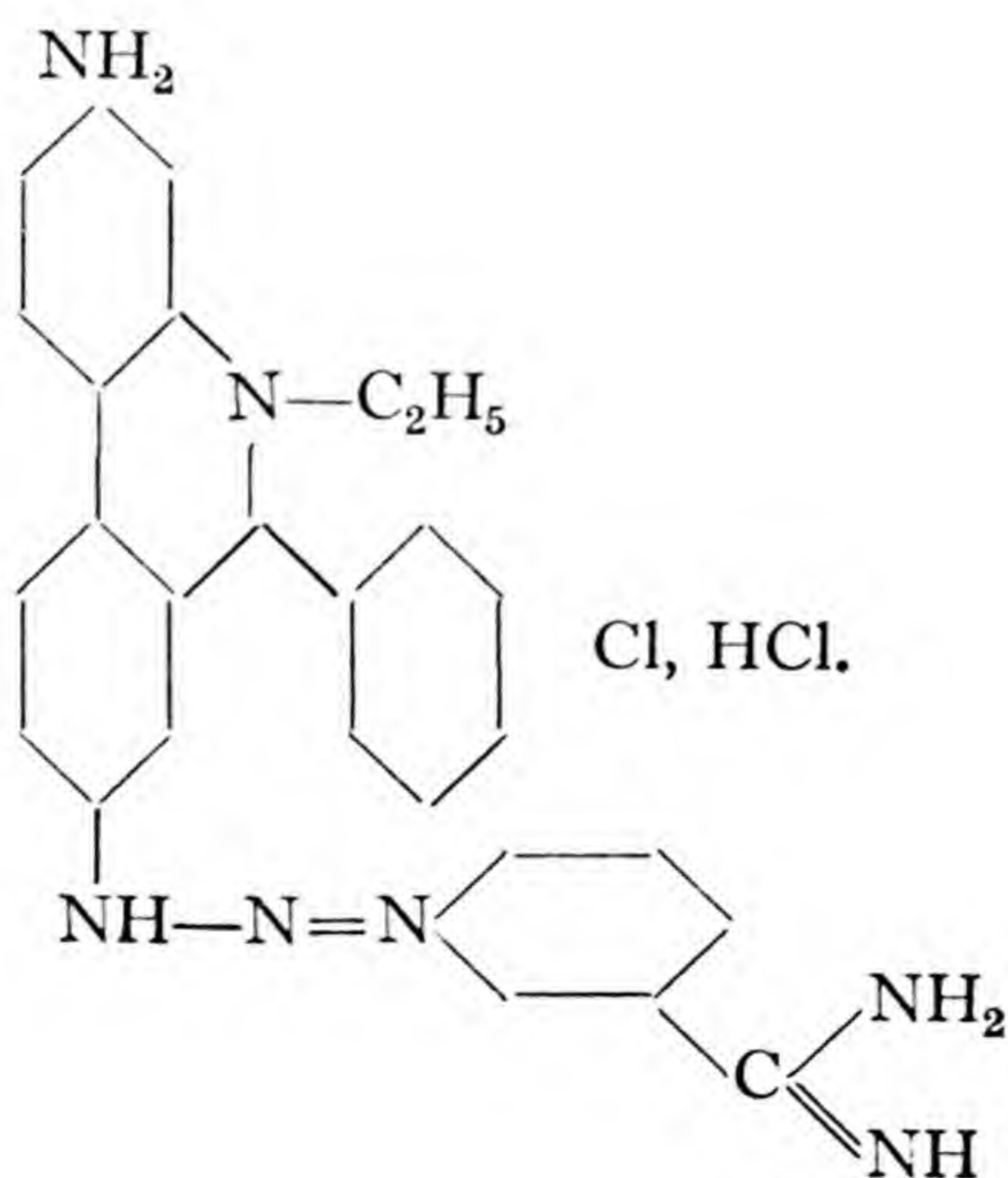
Toxicity. Some at least of the trouble reported with Prothidium may be due to the use of early samples, but care in use, nevertheless, seems necessary. Leach and el Karib (1960) found that a week after inoculation with 2 mg./kg. Prothidium bromide nine cattle had hard swellings at the site of subcutaneous inoculation. These swellings later disappeared. Three out of six animals receiving 5 mg./kg. died 8-9 weeks later in circumstances suggestive of drug toxicity.

Drug resistance. The Kenya Veterinary Department Annual Report for 1960 suggests that Prothidium is the only prophylactic recommended for general field use. Resistant infections were eliminated with Berenil. However, it does appear that Prothidium seems to induce the appearance of resistant strains at a speed which is likely to militate against its general usefulness in the field.

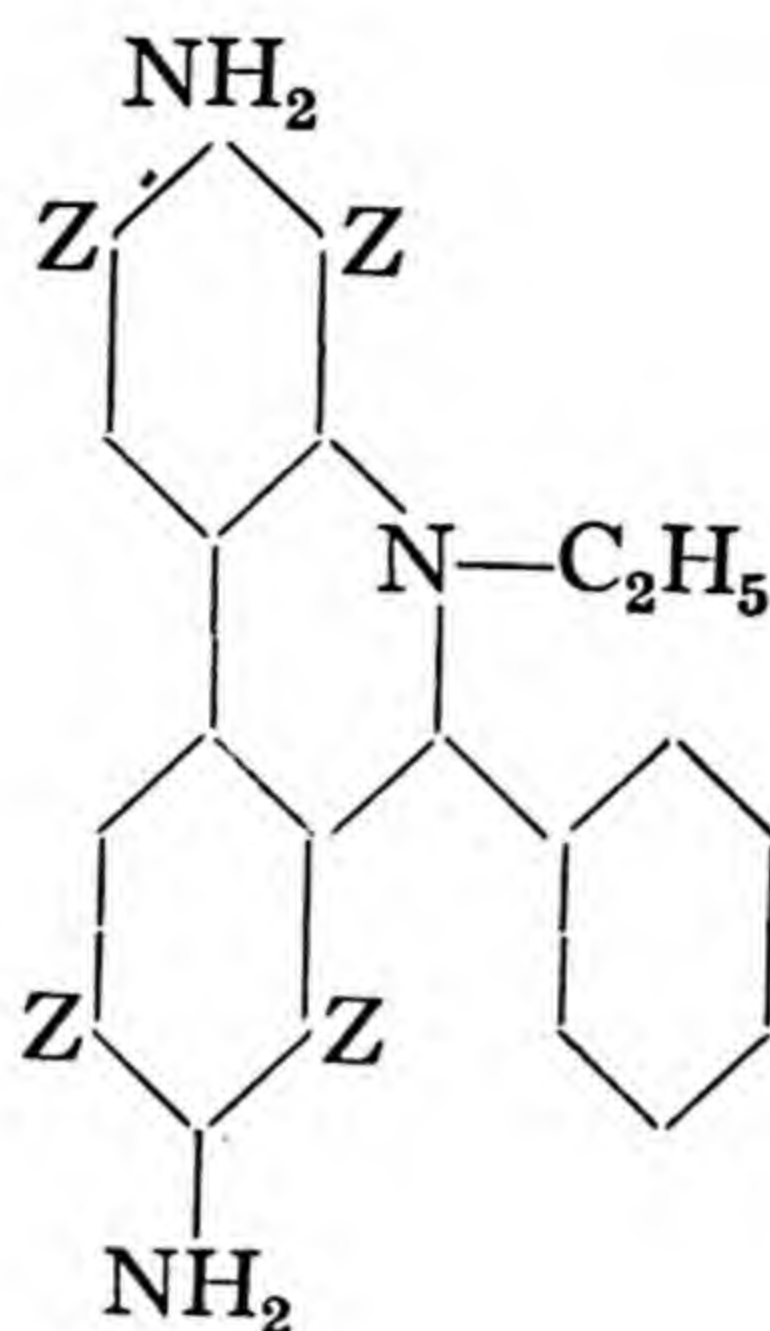
Metamidium

(The *p*-amidino-phenyldiazo-amino derivative of 2-7-diamino-10-ethyl-9-phenyl phenanthridinium chloride (homidium chloride)). Wragg, Washbourn, Brown and Hill (1958). Metamidium is a mixture of two isomers :—

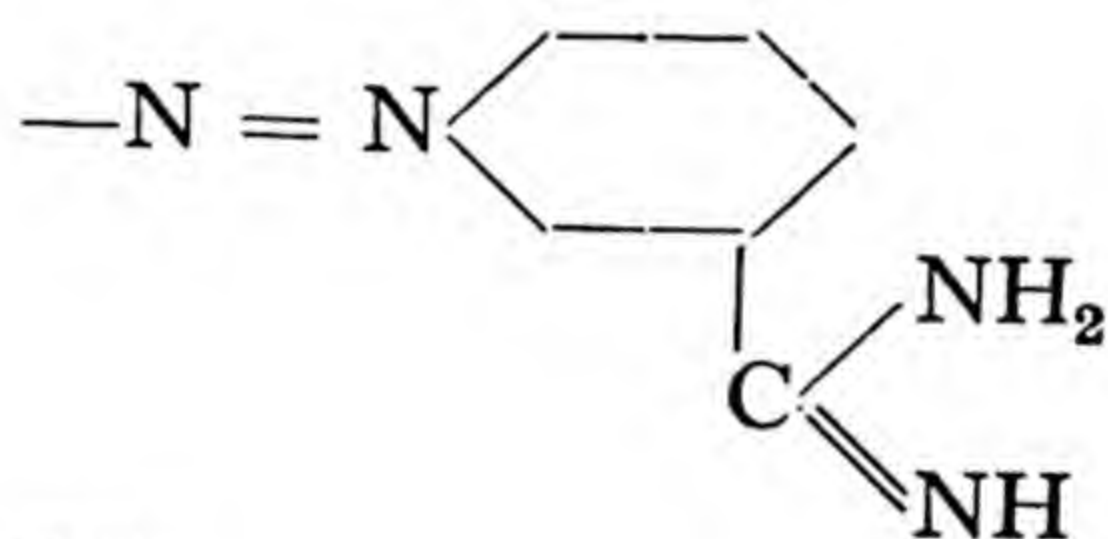
Red Isomer



Purple Isomer



Where one Z =



and the others are (H).

Uses. Metamidium has been used both by itself and as suramin complexes (Berg, Brown and Lucas, 1961). Stephen (1960) investigated the prophylactic and therapeutic effect of metamidium against *T. congolense* and *T. vivax* in cattle and concluded :—

(1) By intramuscular injection metamidium chloride could be toxic for W. African cattle at 10.0 mg./kg.

(2) At 5 mg./kg. i/m there was some weight loss and initial swelling but it was not serious and 204 days' prophylaxis against trypanosomes resulted.

(3) The suramin salt of metamidium gives 111 days' protection at 10 mg./kg. and 128 days' protection at 20 mg./kg.

(4) It is possible that 0.5 mg./kg. may cure animals infected with *T. vivax*.

In a further series of observations on *T. simiae* injected subcutaneously in pigs, Stephen and Gray (1961) found that

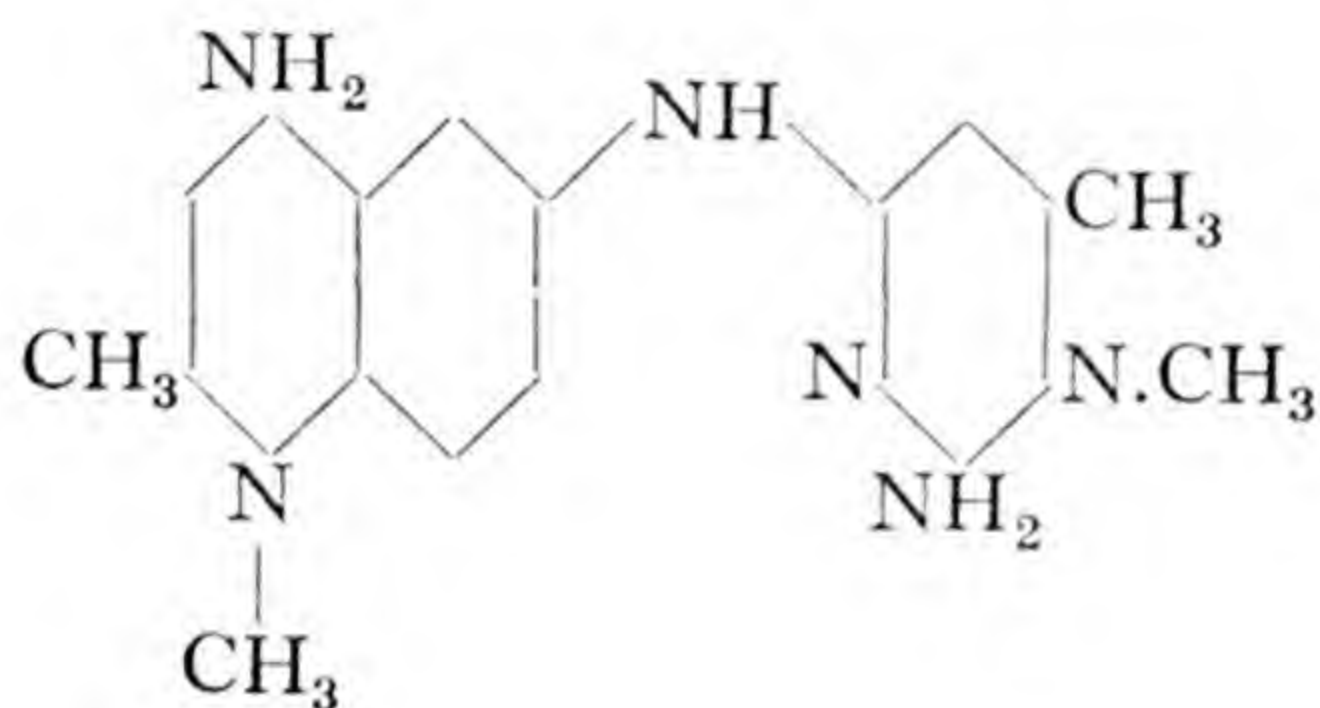
3.0 mg./kg. or 6.0 mg./kg. cleared the blood but fatal relapse occurred in a proportion of cases. Pigs seemed to tolerate the drug better than did cattle.

Related compounds

A related compound (M. and B. 4596) has recently been described (Berg, Brown, Hill and Wragg, 1961).

Antrycide

Description. Antrycide is a complex chemical compound containing a quinoline and a pyrimidine group. The chemical name is 4-amino-6-(2-amino-1 : 6-dimethylprimidinyl-4-amino) 1 : 2-dimethylquinoline.



Methyl Sulphate
 $2(\text{CH}_3\text{SO}_4)^-$

Chloride
 $2\text{Cl}^-, 2\text{H}_2\text{O}$

Physical characters

(a) *Antrycide methyl sulphate*—pale cream in colour, odourless, finely crystalline ; melting with decomposition at 265°C . ; very soluble in water, forming a stable solution. The dry powder withstands sterilisation at 130°C .

(b) *Antrycide chloride*—almost white, finely crystalline ; melting with decomposition at 312°C . ; almost insoluble in water, stable to heat.

(c) *Antrycide pro-salt*—Two formulations have been used under field conditions, the one in use at present (1962) consisting of drug in the proportion of 1.5 gm. methyl sulphate to 1.0 gm. of the chloride.

Uses. So far antrycide has proved effective against certain trypanosomes. It is not known to have any action against other protozoa. Particular activity has been shown against *T. congolense*, *T. evansi*, *T. equinum* and *T. equiperdum*. *T. brucei* and *T. simiae* also are susceptible. *T. vivax* is relatively resistant.

After subcutaneous injection, antrycide methyl sulphate is rapidly absorbed and rapidly excreted. Antrycide chloride remains at the site of injection and is absorbed very slowly. The slow rate of absorption accounts for the prophylactic action.

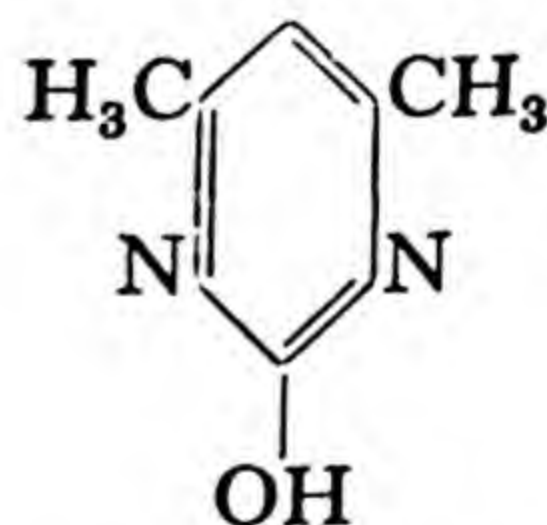
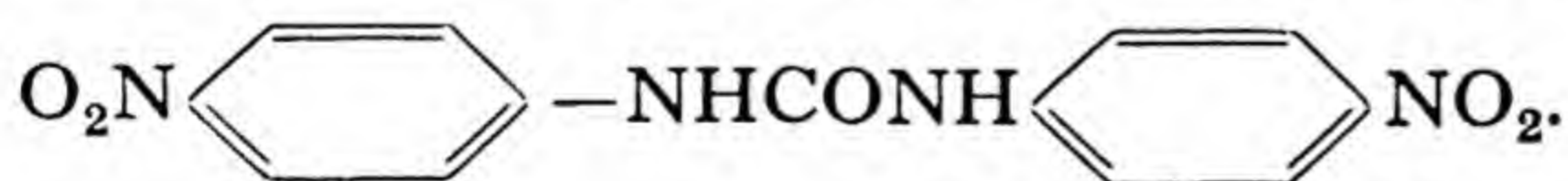
Dose (1), The methyl sulphate. Against *T. congolense* in the field a minimum dose of between 0.5 and 2.2 mg./kg. is needed ; against *T. evansi.*, *T. equiperdum* and *T. equinum* about 3.0 mg./kg. and against *T. vivax* in cattle 4.4 mg./kg. is recommended. The drug is given by subcutaneous injection, *not* intravenously and usually as a 10 per cent. solution.

(2) *The pro-salt.* The pro-salt is used for prophylaxis. The mixed salts are dissolved in water in the proportion, 35 gm. of the dry salt in 150 ml. water. The mixture is then given so that as much methyl sulphate is used as with the curative dosage.

Toxicity. As a rule the drug is safe in use. 5 mg./kg. methyl sulphate is the maximum tolerated dose in African cattle. Horses are much more susceptible to toxicity than are cattle. Broadly speaking, young animals are more susceptible to toxic effects than are old. Treated stock should be handled carefully and kept cool. Hard swellings may develop at the site of inoculation. In addition there may be acute or chronic systemic disturbance. Acute symptoms include twitching of the nostrils and lips, followed by salivation, muscular tremors, distressed breathing and collapse. Chronic toxicity may be associated with dysentery sufficiently severe to cause death.

Nicarbazin

This is stated to be a molecular complex of 4, 4'-dinitrocarbanilide and 2-hydroxy-4, 6-dimethyl pyrimidine.



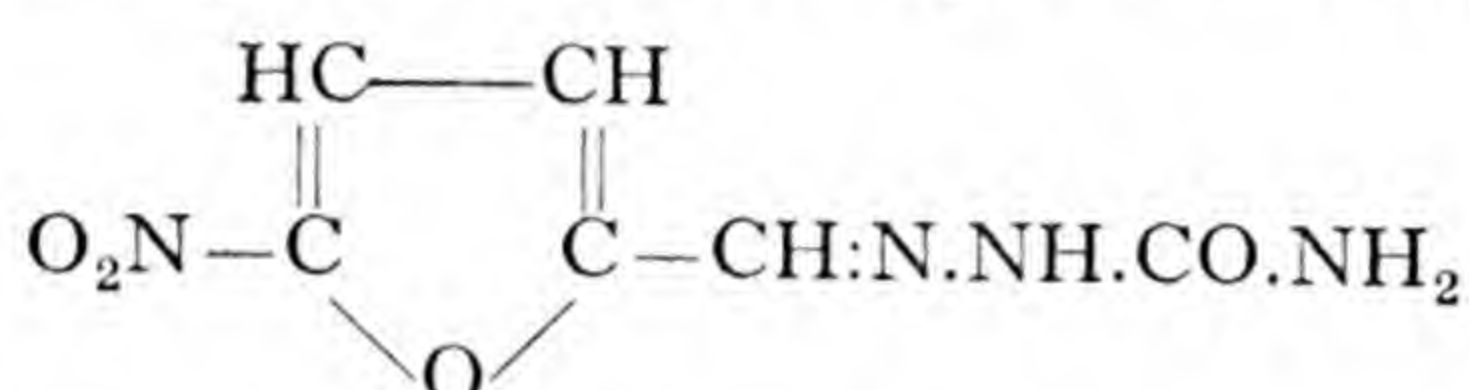
Physical characters. Stated to be a buff-coloured odourless powder but usually supplied only as a premix in a carrier of mixed composition.

Uses. Nicarbazin is used at a concentration of 0.0125 per cent. of the food fed continuously for the control of coccidiosis in chickens. Hammond *et al.* 1958 had little success in the treatment of *Eimeria bovis* in cattle.

Toxicity. The drug is safe for use with growing stock but is contra-indicated for laying hens. Depigmentation of the egg-shell, yolk mottling and reduction in egg size and hatchability have been reported with 0.003 per cent. or more of the drug in the food.

The nitrofurans

Nitrofurazone (5-nitro-2-furaldehyde semicarbazone)



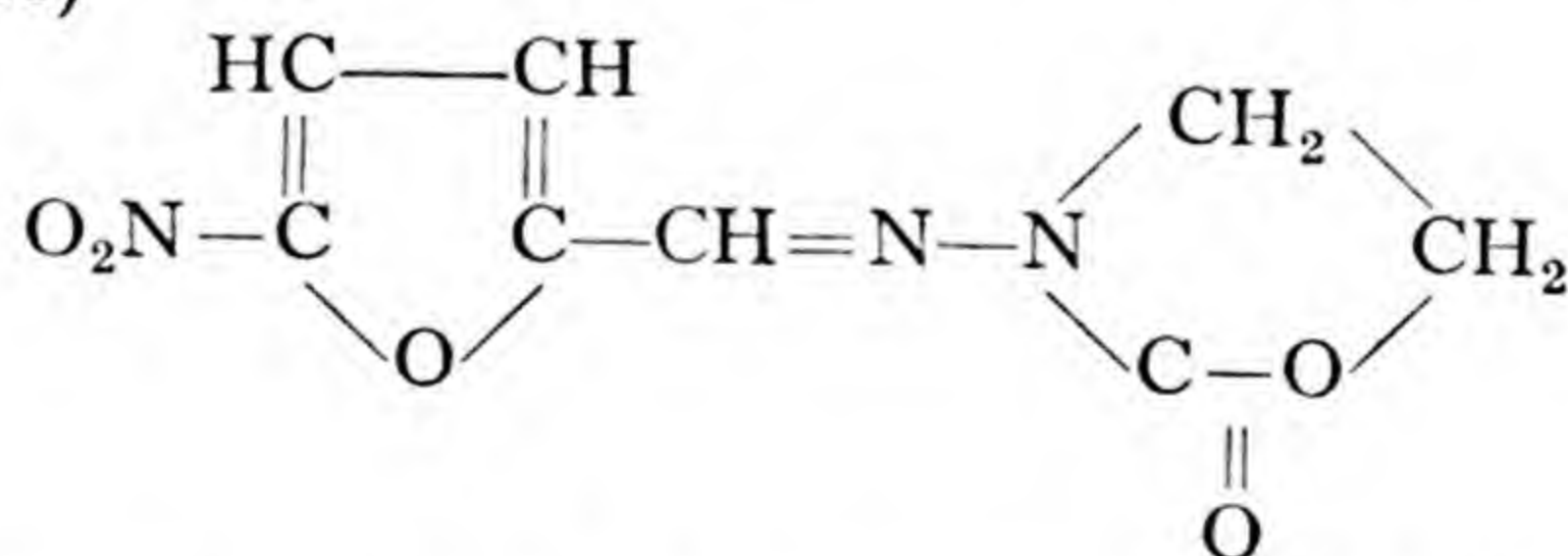
Physical characters. Practically insoluble in water. Crystallises as yellow crystals. The pharmaceutical grade is a light yellow powder. The veterinary grade is greyish-yellow.

Uses. Used for the control of coccidiosis, particularly avian coccidiosis.

Dose. Incorporated in the food at a concentration not exceeding 0.01 per cent. for continuous administration or at 0.02 per cent. for not more than five days.

Toxicity. Retardation of growth is noted at concentrations in the food exceeding 0.01 per cent. when fed continuously. At a concentration exceeding 0.02 per cent., toxicity, with nervous symptoms may occur after ten days' feeding. 0.04 per cent. of the drug in the food for a few days may produce toxicity with death.

Furazolidone (*N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidinone)



Uses. Has been used for the control of *Histomonas* and *Hexamita* in turkeys.

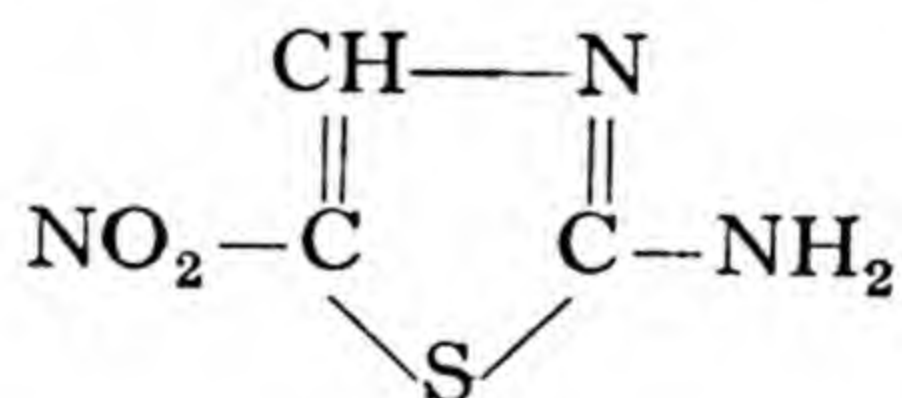
Dose. At a concentration in the food of 0.04 per cent. for up to fourteen days or continuously at 0.01 per cent. of the food.

The drug appears to be well tolerated. Harwood and Stunz (1954) noted some retardation in rate of growth when the drug was fed at 0.33 per cent. of the food for four weeks (possibly the result of a reduced food consumption). The LD/50 for chicks was 500 mg./kg. Cooper and Skulski (1955) found histological changes in the testes of treated birds. It should apparently be used with caution with breeding stock.

Bifuran

This is a mixture of furazolidone and nitrofurazone for which coccidiostatic and growth-promoting properties have been reported. The mixture is fed in the food at a "preventive" level which represents 0.0055 per cent. of nitrofurazone and 0.0008 per cent. furazolidone. A soluble product also is available.

2-amino-5-nitrothiazole (Entramin, Enheptin-T)



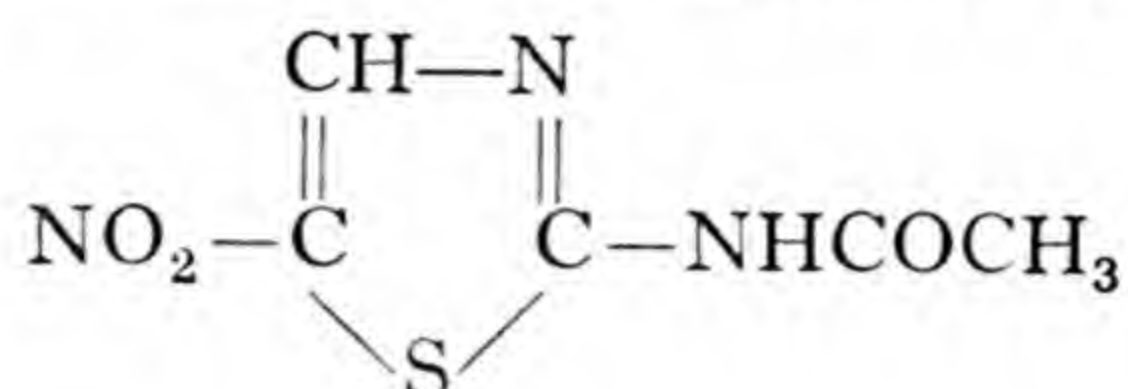
Physical characters. A greenish yellow powder, not readily soluble in water, but soluble in some non-toxic organic solvents.

Uses. This drug is a specific for histomoniasis in turkeys and has also been used against *Trichomonas gallinæ* in pigeons.

Dose. For the prevention of histomoniasis it is fed continuously in the mash at a concentration of 0.05 per cent. For the cure of early cases use 0.1 per cent. in the food for fourteen days followed by 0.05 per cent. for at least another month. Capsules for force-feeding are available and a solution (suspension) has been prepared.

"Entramin" is a 16 per cent. w/v solution of 2-amino-5-nitrothiazole used for the medication of drinking water.

2-acetamido-5-nitrothiazole (2-acetylamino-5-nitrothiazole, acinitrazole, "Entramin A", "Enheptin-A").



This acetamido derivative is a more active compound than 2-amino-5-nitrothiazole and is similarly used against *Histomonas meleagridis*. It is stated to be effective against *Trichomonas gallinæ*.

Physical characters. As compared with 2-amino-5-nitrothiazole the acetamido derivative is insoluble.

Uses. For *treatment* of histomoniasis the drug is given in capsules at the rate of 100 mg./3-6 lbs. body weight on alternate days for five administrations or in the food at 0.05 per cent. for 14 days. To avoid relapse, treatment at the "preventive" level must be continued for at least a month. For *prevention* the drug can be fed continuously at 0.025 per cent. of the food.

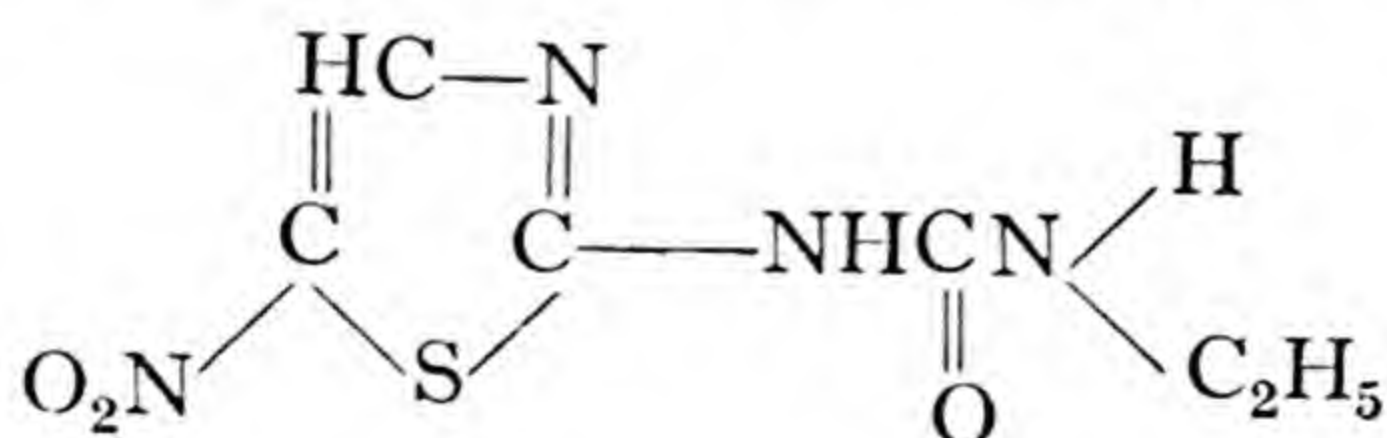
Toxicity. Some loss in weight has been reported when the drug was fed at 0.025 per cent. of the food for 63 weeks. Testicular atrophy has been observed in chickens (Pino, Hudson and Veros, 1955) following continuous treatment.

"Entramin-A" is offered in two forms:—

(1) As a premix containing 22.5 per cent. drug, for incorporation in the food.

(2) In 100 mg. capsules for the treatment of individual birds. (It is fed at a final concentration of 0.025 per cent. of the food for prevention of disease caused by *Histomonas* and at 0.05 per cent. for two weeks for therapy.)

Nithiazide (1-ethyl-3-(nitro-2-thiazolyl) urea, "Hepzide").



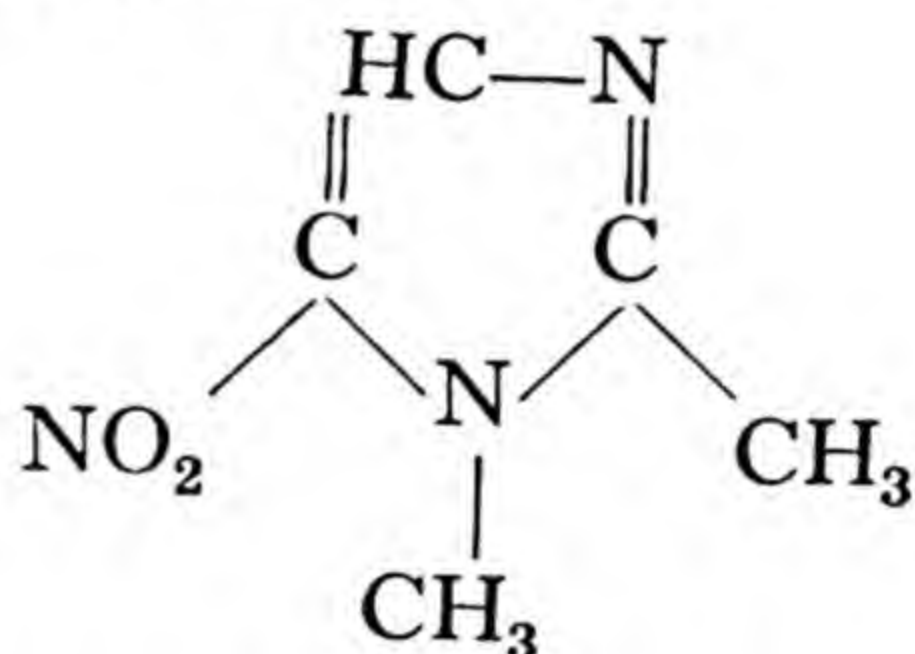
Nithiazide is related to 2-amino-5-nitrothiazole.

Uses. Cuckler and Malanga (1956) found the drug to be effective against *Histomonas* at 0.025 per cent. of the food when treatment started one day before or three days after the day of infection. If treatment were delayed until five days after infection 0.05 per cent. was needed. As with other histomonocidal drugs relapses were likely to occur when the drug was discontinued. Nithiazide can be given in water at concentrations of 0.01 per cent. to 0.04 per cent.

Toxicity. There appears to be no significant toxicity when 0.1 per cent. of the drug is fed in food for 27-62 weeks. When fed in the mash at 0.0125 per cent. to 0.05 per cent. for 62 weeks there was no evidence of toxicity in turkeys (Cuckler, Porter and Ott, 1956).

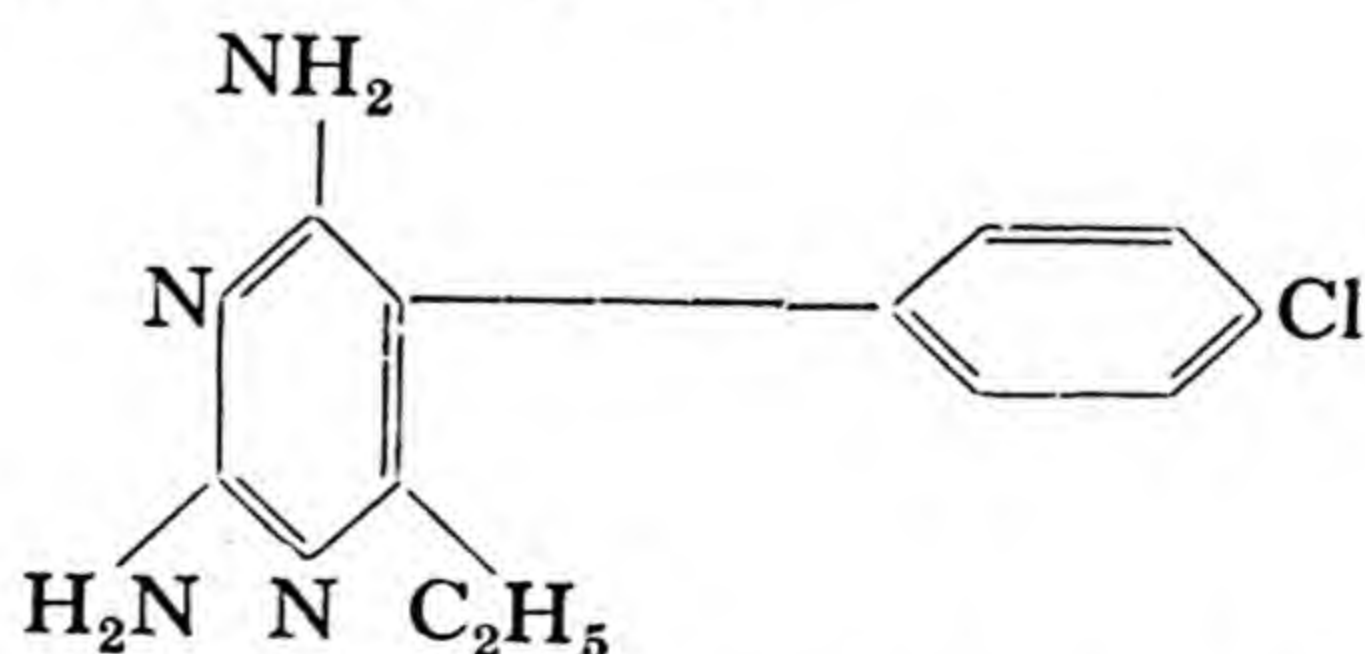
Dimetridazole (Emtryl)

1,2-dimethyl-5-nitroimidazole



This compound, which is an analogue of metronidazole (used for the treatment of *Trichomonas vaginalis*) has been reported by Lucas (1961) to be active against *H. meleagridis* at a concentration of 0.0125 per cent. of the food.

Pyrimethamine (Daraprim)



Uses. In human medicine, pyrimethamine has found important uses against malaria and it has been found effective both in animals and in man against *Toxoplasma*. In very small quantities

it has the power to potentiate the action of the sulphonamides both against *Plasmodium gallinaceum* in birds, and against *Eimeria tenella* in domestic chicks. It is believed to interfere with the folic-folinic acid metabolism and hence with the rapid multiplication of the schizogonous stages of *Sporozoa*.

Mixtures of Pyrimethamine and Sulphonamides are now available commercially for the control of coccidiosis in chickens.

CHAPTER XV TECHNIQUES AND EQUIPMENT

EQUIPMENT

IN addition to the usual laboratory equipment of glassware, bottles and jars, filter papers, grease pencils, Petri dishes, culture dishes, glass pipettes and rubber teats, dissecting equipment and a spirit lamp or Bunsen burner, the following items may be especially mentioned :—

(1) A microscope and accessories

A compound microscope ; objectives 16 mm. = $\frac{2}{3}$ ", 4 mm. = $\frac{1}{6}$ ", and 2 mm. = $\frac{1}{12}$ " ; eye-pieces, $6\times$ and $10\times$; substage condenser ; a moving stage and a microscope lamp with a ground-glass screen ; a micrometer eye-piece and a micrometer slide ; a warm stage apparatus ; McMaster slides ; immersion oil ; 17 mm. square cover-slips (thick) ; $1\frac{1}{2}$ in. \times $\frac{7}{8}$ in. thin cover-slips ; $\frac{7}{8}$ in. square cover-slips and an object marker.

In the tropics it is advisable to clean thoroughly all optical parts and to keep them in a sealed container over some calcium chloride. In a humid atmosphere a film of fungus may otherwise develop on the lenses. Slides and cover-slips need special attention to prevent the growth of fungi and before use should be boiled in a porcelain dish in the following solution :—

Potassium bichromate, 6 gm.

Sulphuric acid, 6 c.c.

Distilled water, 100 c.c.

Boiling in this solution also removes grease. When the container has cooled, the slides or cover-slips should be removed with forceps and washed in running tap water for about thirty minutes to remove all traces of acid. After thorough washing they should again be removed with forceps and stored in a tightly-stoppered jar of methylated spirit.

(2) Other apparatus

A Clayton-Lane centrifuge ; Clayton-Lane centrifuge buckets and tubes ; conical buckets and tubes ; wide mouthed ground-glass stoppered bottles and glass beads ; wire mesh screens in

assorted sizes ; a mechanical blender ; Stoll pipettes ; balances ; a 37° C. and a 26° C. incubator ; a refrigerator and a mechanical counter (hand tally).

(3) Reagents

For details of stains, fixatives and culture media reference should be made to the appropriate sections later in the chapter.

COLLECTION AND EXAMINATION OF MATERIAL

(1) Blood

The skin at the site from which blood is to be obtained is first cleaned with methylated spirit to remove grease or other contamination. With cattle, sheep and rabbits, blood can be obtained from the ear vein ; with mice from the tip of the tail and with birds from the wing vein that passes over the elbow on the under surface of the wing. With birds, feathers on the under surface are plucked, the area cleaned with spirit or ether and then smeared with petroleum jelly. The vein is raised by finger pressure and pierced with a needle, blood being collected from the hollow formed by the elbow and the wing membrane.

Wet preparations are often used for the detection of trypanosomes. A spot of blood is placed on a clean slide, covered immediately with a clean dry cover-slip and examined under the microscope. Even with scanty infections the active movements of the flagellates can usually be seen with the 4 mm. objective. Dark ground illumination can be used when examining blood for living trypanosomes but its effectiveness is limited by the small field under examination when using an oil immersion objective.

In the preparation of fixed and stained slides oxalated blood is quite satisfactory for the demonstration of erythrocytic parasites but it is not so satisfactory for the examination of the white corpuscles. For these, fresh untreated blood is best. In any event, fixation and staining of the smear should be completed as soon as possible. When making the smear it is absolutely essential to use clean grease-free slides and to make the films either thin or very thin, preferably using a special spreading slide. A spreading slide should have a straight flat edge. One corner may be removed in order to get the edges of the film clearly spaced from the edge of the slide. This is because, like the white cells,

parasites in blood tend to be more numerous at the edge of the film. The angle of the spreading slide controls the thickness of the film, a high angle giving a thickly spread smear and a low angle a thin one. For practical purposes the angle of the spreading slide should not be more than 20° . If possible, the slide on which it is proposed to make the film should be placed on a flat hard surface. The blood on the skin is then touched with the under surface of the edge of the spreading slide and so conveyed to the slide on which the film is to be made. The smear is made by pushing the spreader with a smooth steady movement.

After thorough drying the smear is fixed (methyl alcohol 1-2 minutes or ethyl alcohol about fifteen minutes) and stained. Giemsa or Leishman stains are in general use. Undiluted Leishman stain will simultaneously fix and stain.

It may sometimes be possible to concentrate blood parasites. Trypanosomes can be concentrated by the use of the centrifuge. Blood is collected into a sterile tube containing an equal quantity of 1 per cent. sodium citrate solution to prevent clotting. The fluid should be spun in a centrifuge at high speed for 20 minutes and the supernatant fluid removed. Distilled water or 2 per cent. acetic acid is added to the deposit to hæmolyse the red cells, centrifugation is repeated, the supernatant fluid again removed and smears of the deposit stained and examined.

When blood containing *Babesia* is spun there is a tendency for the parasitised corpuscles to be concentrated in the upper layer of the red cells, as their specific gravity is less than that of uninfected cells. This phenomenon should provide a means of concentrating parasites although in practice separation of the blood layers is difficult.

A method of concentrating malaria parasites has been described by Rogers (1946) and has given satisfactory results in practice. The method depends on the fact that infected cells become concentrated in the upper layers of the red corpuscles (as with *Babesia*). The cells are collected from that layer into a capillary tube from which they are driven by a second centrifugation into a pipette.

(2) **Fæces and intestinal contents**

All containers used for specimens must be thoroughly cleaned. Great care must be taken to ensure that all traces of chemicals

used in the cleaning are removed as the success of the technique often depends on the organisms being still alive.

Fæces are best collected from the rectum as this avoids the danger of contamination from the litter. When blood or mucus are present these should be separated for immediate examination. It must be remembered that many of the organisms occurring in the intestinal tract are very liable to become damaged when once removed from their normal environment (*Hexamita meleagridis* is particularly delicate and it is essential to obtain material from a freshly killed bird). Many organisms lose motility as soon as the temperature is reduced so that emulsions of fæces or intestinal contents should be made in warm normal saline and it may be necessary to maintain the emulsion at the appropriate temperature on a warm slide. Direct microscopical examination of fæces or gut contents is often of value, particularly if there is a swarming infection. Motile organisms can be protected from distortion by supporting the cover-slip or by the use of a hanging drop in a cavity slide.

With more robust organisms, preliminary screening of the material followed by flotation and/or sedimentation can be used for concentration. Eosin stains fæcal debris an intense red but freshly passed protozoa, including cysts, resist the stain and are thus easily identified as white bodies, even with the 16 mm. objective. The chromatoid bodies of *Entamæba histolytica* cysts are rendered more apparent in eosin solutions.

The flotation method of concentration

The principle of this method is to bring the specific gravity of the fluid of the suspension to a point at which it is less than that of the particles of debris but greater than that of the material which it is wished to collect. Various solutions, including saturated sodium chloride, sugar and zinc sulphate solutions can be used according to the type of material which it is wished to isolate. The material should never be left in contact with the flotation fluid for longer than is necessary. The fæcal suspension, after emulsifying in water, is screened by sieving to remove gross particles, the flotation agent is added and the mixture spun at 1500 to 3000 revolutions per minute in order to deposit the debris and to bring the protozoa to the surface. They can be collected either by

first covering the specially ground top of the centrifuge tube with a cover-slip to which the parasites adhere during centrifugation or (at the end of the process) with a wire loop or a cover-slip carefully lowered onto the surface film. Both flotation and sedimentation are used in the collection of oocysts from fæces.

The use of saturated salt solution for flotation

Collection of oocysts from fæcal samples. A suitable quantity of the fæcal sample is placed in a glass bottle along with a quantity of glass beads, shaken vigorously, and the resulting emulsion is passed through a fine screen, the filtrate being caught in a bowl. The screen is then washed thoroughly and the washings also caught in the bowl. The filtrate and washings are poured into large jars and allowed to sediment for several hours, preferably overnight. After use, the bottle, beads, screen and bowl are thoroughly cleaned by vigorously washing under the tap and placed ready for use when required on a future occasion. After sedimentation of the oocysts is complete, the supernatant fluid is siphoned off until only two or three inches of fluid are left in the bottom of the jars. This residual suspension is poured into centrifuge tubes and spun for two to three minutes at about 1500 r.p.m. The supernatant fluid is then poured off and each tube is filled with saturated common salt solution. The tubes are placed in the centrifuge and spun as before, and on this occasion the oocysts will float to the top of the saturated salt solution. After flotation, the top quarter of the salt solution is poured into a large jar of water and the oocysts are allowed to sediment overnight. The supernatant fluid is siphoned off the following morning and the residue again spun in the centrifuge and the supernatant fluid poured off from each tube. The residue at the bottom of each tube contains oocysts and should be shaken up with 2 per cent. potassium bichromate solution and poured into Petri dishes ; the depth of fluid in the Petri dish should never exceed half an inch.

The use of zinc sulphate solution for flotation

This method has been used for the concentration of the cysts of *Entamoeba*. Faust *et al.* (1939) used a solution with a specific gravity of 1.180. A solution of specific gravity 1.280 will bring

up heavier cysts but will also, of course, bring up more debris. The specimen is emulsified in water, strained through a sieve and centrifuged. The supernatant fluid is pipetted off, the zinc sulphate solution added and the suspension mixed by agitation, care being taken to avoid the formation of bubbles. The material is then centrifuged and a specimen of the surface film removed for examination. To these specimens eosin or Gram's iodine can be added. All material should be examined as soon as possible as the cysts soon collapse in the zinc sulphate solution.

The use of sugar solution

Sugar solutions may be used similarly.

Isolation of single oocysts

For some purposes it may be necessary to isolate single oocysts. This can be accomplished by the use of a micropipette or by such a technique as (a) dilution of the material onto agar plates, (b) examination for oocysts with a microscope and (c) cutting out the appropriate section containing the oocyst. It must be remembered that the precise species identification of a single oocyst is practically impossible.

(3) Examination of the genital tract for *Trichomonas foetus*.

(a) The diagnosis in the bull

At the present time there is no absolutely certain way of detecting infection in the bull. There is, however, a reasonably good chance of finding the parasite by direct microscopical examination. There is a better chance of isolating the parasite by cultural methods.

Technique for making preputial washings

Apparatus. The apparatus consists of a 150 ml. glass bottle or flask, a "Wellcome" flutter valve injection apparatus and a glass end-piece for insertion into the preputial orifice. This can be made by cutting the end from a 6 in. \times $\frac{5}{8}$ in. test tube, rounding the end in a Bunsen flame and inserting a rubber bung containing a glass tube to which the end of the rubber tubing of the injection apparatus is attached. The whole apparatus, with the exception of the flask, should be sterilised by boiling.

Preparation of saline for preputial washings

T. foetus is a very delicate organism. It is essential to use saline at an exact concentration of 0.85 per cent. It is important also that only glass-distilled water should be used in the preparation of the saline. Tap water or water distilled in metal stills is very likely to immobilise the parasite.

Thirdly, it is important to *ensure that the solution is sterile*, as saline, tap water or even distilled water which has been stored may all contain motile protozoa.

Collection of the samples

Restraint should be directed towards preventing the bull moving over onto the operator, and preventing forward kicking with the near hind leg. One assistant controls the bull's head, which should be firmly secured and, in the case of a right-handed operator, the animal placed with his right flank against a solid fence, or against the wall of the loose-box.

A second assistant should be instructed to keep the weight of his shoulder into the bull's left flank just in front and slightly above the stifle joint. This serves to prevent the animal moving onto the operator, and to break the force of any forward kicking. Further restraint may be applied but is not recommended as a routine.

The bottle containing 100 to 150 c.c. of saline is placed for a few minutes into hot water, before taking the washing, in order to raise the temperature of the saline to 30°-35° C. The glass end-piece is placed well into the preputial orifice which is then held closely around the tube by digital pressure using the left hand. The assistant is directed to raise and invert the container so that the saline flows into the preputial cavity. The fluid which has gravitated into the prepuce is vigorously massaged so as to wash the mucous surfaces, the left hand retaining the glass end-piece and preventing any undue escape of saline. The container is then lowered and the fluid massaged out of the prepuce and back again into the container. Washings of the prepuce should not have been made within the previous seven days nor should the bull have been used for service or the collection of semen.

Clipping off the preputial hair is usually unnecessary and the

use of detergents which might immobilise the trichomonads is to be avoided.

Description of Trichomonas fœtus

In the unstained preparation, only the following structures can be distinguished: the undulating membrane, the posteriorly projecting part of the axostyle, the presence of anterior flagella (the actual number of which is usually not distinguishable) and a round or oval refractile body marking the position of the nucleus. The posterior flagellum is very infrequently observed.

Leucocytes are usually seen among the epithelial debris and these will give some indication of the size of the trichomonad, the diameter of the leucocyte being slightly less than the overall length of the trichomonad, excluding the flagella and the portion of the axostyle extending posteriorly beyond the protoplasm of the "body".

In an unfavourable environment, the trichomonad may tend to become spherical, in which case it would appear slightly bigger, more refractile and less granular than a leucocyte.

Examination of washings

This should be carried out at the earliest convenience and not more than 4 hours after collection. The method is to centrifugalise at about 2000 r.p.m. for five minutes; a square coverslip is then placed over a drop of the deposit on a slide and the examination carried out methodically by the aid of a mechanical stage. Preliminary examinations are best carried out under the 16 mm. objective with a high-power eye-piece ($\times 15$). Any motile body or movement of the cellular debris should be examined under the 4 mm. objective. If the cause of the movement appears to be a trichomonad the *undulating membrane must be detected*. Further organisms should be looked for under the 16 mm. and the procedure repeated on the detection of further movement.

Movement observed in the washings may be caused by motile spermatozoa, motile bacteria, Brownian movement, or by free-living flagellates and ciliates. Some of the free-living protozoa bear a close general resemblance to *Trichomonas fœtus* and it is important to be fully aware of the possibility of their occurrence in preputial washings from bulls. The detection of an undulating

membrane is of the greatest diagnostic importance in the recognition of *T. fætus*.

For the preparation of cultures, samples of 0.1 to 0.5 ml. of the centrifuged deposit are inoculated into test tubes of glucose-broth serum medium containing antibiotics. The cultures should be incubated at 37° C. and examined for the presence of trichomonads at intervals of two to three days for about ten days. For this purpose wet cover-slip preparations should be made with samples of the culture medium. All the precautions outlined above for the identification of trichomonads should be observed.

Direct examination for the active parasites should be made as soon as possible after collection but experience has shown that in many cases the parasites remain alive for at least 24 hours although motility is diminished. It is, therefore, possible to prepare cultures from preputial washings after a delay of not more than 24 hours, thus allowing washings to be sent to the laboratory for this test through the post.

(b) **The diagnosis of Trichomoniasis in the female**

Two methods are available for the diagnosis of trichomoniasis in the female. The parasites themselves may often be identified in vaginal discharges or specific agglutinins to *T. fætus* may be demonstrated in the vaginal mucus. Both of these tests give variable results in an animal known to be infected and examinations for this disease in the female must, therefore, be applied on a herd basis in the expectation that where the disease exists at least a few animals will give positive results.

Collection of vaginal discharge

For this purpose glass pipettes 18 in. long and $\frac{3}{8}$ in. diameter are used. Some operators prefer to have the tube bent 5 in. from one end so that the angle between the two arms of the tube is about 150°. These tubes can readily be prepared and sterilised in large numbers and one tube should be used for each animal.

In use, the tube is inserted into the vagina and a mouth-piece consisting of a suitable length of rubber tubing and a short length of glass tubing is attached. Mucus from the vagina is then aspirated into the tube which is then plugged at both ends with

cotton wool and then taken back to the laboratory for examination.

Examination of mucus for Trichomonas foetus

As with preputial washings, the parasites may be detected by direct microscopical examinations. In pyometra cases the flagellates are nearly always present. In other cases the parasites are present in the vagina just after abortion, or, in later stages of the disease, just prior to œstrus.

Cover-slip preparations may be made by mixing a loopful of mucus in a drop of physiological saline and covering with a cover-slip. Microscopical examinations should be carried out as for preputial washings.

A small quantity of mucus may also be used as an inoculum for Glucose-broth-serum medium.

Because of the possible presence of antibodies in the mucus it is important that mucus should be examined as soon as possible after collection.

(4) Examination of tissues

Tissues believed to contain protozoa can be examined by making smears or impression preparations or after cutting sections. The organ to be examined is removed from the body and a portion cut off with a sharp knife, care being taken not to squeeze the tissue more than is necessary. Smears are made by drawing the cut surface along a clean slide. Impression preparations are made by gently pressing the tissue onto a slide three or four times. A series of slides should be made because the earlier preparations tend to contain a large quantity of blood but little tissue. As the surface blood is smeared off, a greater proportion of tissue cells will be present. The preparations are stained with Giemsa or Leishman stains. It is usually advisable to stain an impression smear more deeply than a blood smear.

For the preparation of sections of tissue, the material must be cut into thin slices and fixed in a large volume of fixative. It is essential for fixation to be rapid and complete. Bouin's fluid, and 10 per cent. formalin are in common use as fixatives. Other fixatives are used for special techniques. When properly fixed the block of tissue is embedded in paraffin wax and the sections are cut.

FIXATION AND STAINING

Recommended fixatives*Ten per cent. formalin in normal saline*

This is a good general fixative in which tissues can be left for several days although fixation is complete in 12-24 hours, depending on the bulk of the material. After fixation, the tissue is washed while being taken up through the alcohols.

Bouin's solution

This is very good for nuclear staining but it causes tissue distortion. Penetration is rapid.

Formula : Picric acid saturated aqueous solution . 30 pts.
 40 per cent. formaldehyde . . . 10 pts.
 Acetic acid 2 pts.

Prepare freshly immediately before use.

Carnoy's fixative

This is particularly recommended for use before Shortt and Cooper's stain.

Formula : Absolute alcohol (ethyl) . . . 6 pts.
 Chloroform 3 pts.
 Glacial acetic acid 1 pt.

Schaudinn's fixative

This is particularly recommended for the fixation of gut contents containing such organisms as *Hexamita*.

Formula : Absolute alcohol (ethyl) . . . 1 vol.
 Saturated mercuric chloride . . . 2 vols.
 plus 5 per cent. acetic acid.

Zenker's fluid

Stock solution : Potassium bichromate . . . 2.5 gm.
 Sodium sulphate 1.0 gm.
 Distilled water 100 c.c.

Just before use add

Glacial acetic acid 5 c.c.
 Mercuric chloride 5 gm.

Recommended stains

This list includes a very small proportion of the numerous excellent stains listed in the specialised text-books on the subject.

Leishman's stain

The staining properties of Leishman's stain in solution in methyl alcohol vary greatly with the age of the stain. To obtain uniform results fresh stain should be made up from powder every month or so. The stain consists of 0.15 gm. Leishman's powder dissolved by grinding in a mortar with 100 ml. pure methyl alcohol. The final solution should be filtered. Thin blood films are allowed to dry thoroughly before being flooded with a measured volume of stain. After about thirty seconds two volumes of neutral distilled water are mixed with the stain on the slide. After twelve minutes the stain is washed off with distilled water and left in a vertical position to drain and dry.

Giemsa's stain

This stain is excellent for blood films and tissue impressions. Like Leishman's it deteriorates to some extent with age, particularly in hot climates but in general it is far more stable. It is usually purchased ready made up in solution. Fresh supplies should be purchased at frequent intervals. Precipitation of stain is a common technical difficulty which can be avoided to a great extent by staining upside down or by making the smear on the cover-slip which is then floated on the surface of the stain. After initial drying, fixation must be carried out in methyl alcohol for three minutes or more. Staining can be by the short or long methods.

Short method. Dilute the stain 1 in 10 parts of water preferably buffered to 7.0-7.2 pH and stain for about half an hour. Blot and air-dry.

Long method. Dilute 1 in 50 parts of buffered water and stain overnight.

Giemsa staining of sections

For Zenker fixed tissue good results are given by a method described by McNamara (1933). The section is treated with 5 per cent. Lugol's iodine in distilled water for twenty minutes, placed in 95 per cent. alcohol, then washed in tap water, treated with 0.5 per cent. sodium hyposulphite for ten minutes, and again washed. Stain for one hour in Giemsa stain 2.5 c.c., acetone 2.5 c.c., methyl alcohol 2.5 c.c., 0.5 per cent. sodium carbonate 2 drops, distilled water 25 c.c. Wash rapidly in tap water and then differentiate

in colophonium 15 gm. and acetone 100 c.c. Then wash in a mixture of acetone 70 c.c. and xylol 30 c.c., clear in xylol, and mount.

A simpler method of Giemsa staining of preparations fixed in Zenker's solution or one of the other mercuric fixatives, consists in treating the section with Lugol's iodine solution, washing in 0.5 per cent. sodium hyposulphite and then staining face downwards for 12 to 24 hours in :—

Giemsa stain	1.5 c.c.
Methyl alcohol	1.5 c.c.
0.5 per cent. sodium bicarbonate	2 drops
Distilled water	50 c.c.

Wash briefly in distilled water, and as the section will be over-stained, differentiate in 95 per cent. alcohol. Dehydrate by passing through solutions of 5, 30 and 50 per cent. acetone in xylol. Clear in pure xylol and mount in cedarwood oil or some other neutral medium.

Sections do not stain uniformly by this method, parts will be over-stained, and the nuclei are not so distinct as by McNamara's method.

Ehrlich's hæmatoxylin and eosin stain

This is an excellent general stain for tissues.

Formula for Ehrlich's hæmatoxylin :—

Hæmatoxylin	2 gm.
Absolute ethyl alcohol	100 ml.
Glycerol	100 ml.
Distilled water	100 ml.
Glacial acetic acid	10 ml.
Potash alum (K_2SO_4 $Al_2(SO_4)_3$ 24 H_2O)	3 gm.

Dissolve the hæmatoxylin in the alcohol and then add the other ingredients. Ripen by exposure to air and sunlight in a paper-capped vessel for at least six weeks, shaking the bottle daily to accelerate the oxidation.

Staining method. From absolute alcohol, stain in Ehrlich's hæmatoxylin for thirty minutes.

Rinse off excess stain in tap water until the sections become blue (two-three minutes).

Differentiate in 1 per cent. acid alcohol until pale pink.

Blue again in tepid tap water.

Transfer to 2 per cent. aqueous solution eosin for ten minutes.

Wash out excess eosin in running tap water.

Dehydrate, clear and mount.

Heidenhain's iron hæmatoxylin stain

This is particularly good for nuclear staining and hence for the demonstration of schizonts. Sections of tissue are placed in a 2-5 per cent. solution of iron ammonium alum in distilled water and left for at least fifteen minutes. They are then rinsed in distilled water and placed in 0.5 to 1 per cent. pure hæmatoxylin in distilled water containing 10 per cent. alcohol, for a quarter of an hour or longer. They are then washed in water and differentiated in the iron-alum solution until nearly decolorized. The sections should be washed from time to time in tap water and observed under the microscope to check the rate of differentiation. After differentiation they are washed for a few minutes in running water, dehydrated and mounted in the usual way.

Both the mordanting in iron alum and the subsequent staining with hæmatoxylin can with advantage be prolonged for several hours.

Shortt and Cooper's technique

The following modification of Giemsa's staining technique will be found useful for such parasites as *Histomonas* when in cæcal tissue.

(1) Results are best when tissues are fixed in Carnoy's fixative for 3-4 hours and are then transferred to absolute alcohol.

(2) Embed in paraffin wax and cut sections. Remove wax and bring the sections down to tap water.

(3) Stain for at least an hour in

Giemsa stain	10 ml.
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Acetone	10 ml.
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Methyl alcohol	10 ml.
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Buffered distilled water (pH 7.2-7.4)	100 ml.
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(4) Wash rapidly in tap water.

(5) Differentiate in 15 per cent. colophonium resin in acetone. Check under low power.

(6) Wash in acetone : xylol 70 : 30

(7) Clear in xylol

(8) Mount in green Euparal.

(*N.B.*) Euparal is an excellent mounting medium. It is completely neutral and blood films will keep their colour for years.

The staining of intestinal protozoa

As a routine method, cover-slip films are made, fixed in Schaudinn's solution for twenty minutes (saturated solution of mercuric chloride 2 parts, absolute alcohol 1 part, glacial acetic acid a few drops), and treated as advised in the section on the preservation of faecal material. They can then be stained in a mixture of 1 part of $3\frac{1}{2}$ per cent. iron alum in distilled water, and 9 parts 50 per cent. alcohol, leaving the cover-slip face down overnight. Wash in 70 per cent. alcohol to remove excess of iron alum, and stain face downwards for 12 to 24 hours in 1 part of Heidenhain's hæmatoxylin and 9 parts of 70 per cent. alcohol. Differentiate next day in dilute iron-alum, wash in 70 per cent. alcohol, dehydrate through increasing strengths of alcohol, clear and mount.

Excellent results can be obtained by using phosphotungstic hæmatoxylin as described by Mallory and Wright, the films being placed in the stain overnight in the incubator at 37° C. after sealing the container to prevent evaporation. Mayer's acid hæmalum also gives good results. Whichever of these methods are used the smears must be passed through grades of spirit to absolute alcohol before clearing and mounting.

For the staining of coccidial oocysts, smears are fixed for five to ten minutes in warm acetic acid. They are then stained with Janus green 1 : 1000 for 10 minutes, washed in water, stained with concentrated eosin, again washed and examined in water.

The preservation of material

Preservation of material may be required either for despatch to other laboratories or for retention as specimens for museum or teaching purposes.

Tissues

Tissues or organs which it is wished to keep for record, or as demonstration material, require to be fixed, for which the simplest

agent is a 10 per cent. solution of formalin. This has the disadvantage of spoiling the natural colour and Kaiserling's method, which preserves the colour, is preferable.

The specimen is first immersed in solution No. 1, consisting of formalin 200 c.c., potassium nitrate 15 gm., acetate of potash 30 gm., and water 1000 c.c. The time required for fixation depends on the size of the specimen, and will vary from about 2 to 24 hours. To prevent the specimen adhering to the receptacle it should be wrapped in cotton wool. The specimen is then drained and placed in methylated spirit till the colour returns. It is then placed in the second solution (potassium acetate 200 gm., glycerine 400 c.c., and water 2000 c.c.) in which it is mounted. This No. 2 solution is made by dissolving the potassium acetate in water, filtering, and then adding the glycerine. It is also advisable to add 1 per cent. thymol to prevent the growth of moulds.

For preservation for histological examination it is best to divide the specimen into small pieces, and immerse them in 10 per cent. formalin in physiological saline, or in Zenker's solution.

Fæcal material

Fæcal material which it is wished to preserve or to forward for examination can be kept in 15 per cent. formalin in saline, but for flagellates or amœbæ it is better to make smears on No. 1 cover-slips and immerse them face downwards in Schaudinn's fluid for twenty to thirty minutes. After fixation, rinse the smears in 50 per cent. spirit and immerse them in a dish of 50 per cent. spirit which has been tinged brown with Gram's iodine (to remove mercury salts). The smears are then transferred to a dish of absolute alcohol for one hour and finally preserved or despatched, packed in cotton wool in a well-stoppered bottle containing 70 per cent. alcohol.

Fæcal specimens to be sent to a laboratory for examination for coccidiosis should be preserved with 2.5 per cent. formol-saline or in 2 per cent. potassium bichromate solution.

Blood films and tissue smears

For examination these can be sent unfixed if they will reach their destination in a few days. Thick blood films should be stained before despatch, and thin blood films which may be some

time in reaching their destination should be fixed, if not stained, before despatch, otherwise the blood undergoes decomposition, and becomes difficult to stain. For demonstration purposes, smears should be covered with a No. 1 cover-slip, using a reliable neutral Canada balsam or Euparal, as acid balsams decolorize them.

Counting techniques for protozoa

Counting oocysts. Four techniques are described below: two are modifications of the McMaster method and two are modifications of the Stoll method. These methods will not indicate the presence of oocysts if less than 100 per gm. are present.

(a) *McMaster technique* (modified and no centrifuge required)

Apparatus required:—

- (1) Special double counting slide.
- (2) A 4 oz. wide mouthed glass stoppered bottle with a mark at the level of 42 ml. and another at the level of 45 ml. or else a measuring cylinder to measure these volumes.
- (3) Glass beads or lead shot, a screen or coffee strainer and a small spatula.
- (4) A balance and weights capable of weighing 3 gm. The McMaster slide consists of two chambers each having an area of more than 1 sq. cm. and a depth of 1.5 mm. The under slide constitutes the floor of the chamber and on the under side of the upper slide two squares are etched, each of an area of 1 sq. cm. In use, the suspension to be examined is run into each chamber until full and then each marked area is examined under the microscope and the number of oocysts counted.

The technique is:—

(1) Fill the 4 oz. bottle with water up to the 42 c.c. mark and add the glass balls.

(2) Weigh out 3 gm. of faeces, stirring the sample well if fluid and place in the glass bottle.

(3) Fit the stopper to the bottle and shake until the faecal matter is broken down.

(4) Pour the mixture of water and faeces through a screen, collect the liquid and discard the debris on the screen.

(5) Add 45 c.c. saturated common salt solution to the strained fluid, mix well and *immediately* withdraw fluid with a pipette and fill the chambers of the McMaster slide.

(6) Count all the oocysts in the two separate centimetre squares. To obtain the number of oocysts per gm., the total number in the squares is multiplied by 100.

(b) *Stoll counting method* (no centrifuge required)

Apparatus required :—

- (1) A pipette to measure accurately 0.15 c.c.
- (2) A 4 oz. wide mouthed glass stoppered bottle with a mark at the 42 c.c. level.
- (3) Glass beads or lead shot.
- (4) A screen or coffee strainer, a small spatula.
- (5) A small balance to weigh 3 gm.

The method is as follows :—

(1) Fill the 4 oz. bottle up to the 42 c.c. mark with tap water and add the glass balls.

(2) Weigh out 3 gm. of faeces stirring the sample well if fluid and place in the glass bottle.

(3) Fit the stopper in the bottle and shake till the faecal matter is broken down.

(4) Pour the mixture through a coffee strainer or screen and catch the strained fluid in a bowl. The debris left on the screen is discarded.

(5) Agitate the filtrate of faeces by stirring and remove 0.15 c.c. of the suspension with the special measuring pipette.

(6) Eject the measured quantity of fluid onto a microscope slide and cover with a $\frac{7}{8}$ in. square cover glass. Although 0.15 c.c. of fluid forms a rather large volume on a slide and may appear to be too much for proper examination under the size of cover glass recommended, there is no real objection to a little liquid running out from under the edge of the cover glass. Cysts must be counted in that part of the fluid not under the cover glass as

well as that beneath the cover glass. If desired, this spreading may be controlled by using a larger cover glass, which increases the area to be searched under the microscope or by drawing two parallel lines the width of a cover glass apart across the slide with a grease pencil or a piece of paraffin wax. These greasy marks prevent lateral spread of the fluid and confine it under the area of the cover glass.

(7) Examine the whole of the 0.15 c.c. sample of suspension under the microscope and count all the cysts seen.

(8) The figure obtained from the count (*i.e.* the total number of cysts present in the 0.15 c.c. of diluted faeces) is multiplied by 100 to give the number of cysts per gm. of the original faecal sample.

Where a centrifuge is available the counting methods given under (c) and (d) should be used in preference to those given above (a) and (b). The latter methods give cleaner preparations with less debris on the slide and the cysts are therefore much easier to see.

(c) *McMaster counting method* (second modification—centrifuge required)

Apparatus required as for (a) but the second bottle marked at 45 c.c. is not required.

Method. Steps (1) to (4) are carried out as under (a) and then proceed as follows:—

(5) Pour a composite sample of the strained fluid into a centrifuge tube not filling the tube quite to the top and spin for two minutes at about 1500 r.p.m. Remove the tube from the centrifuge and pour off the supernatant fluid.

(6) Agitate the tube pendulum-wise until all the sediment is loosened and forms a liquid mud at the bottom of the tube and then fill the tube with saturated common salt solution to the same level as before.

(7) Thoroughly mix the contents of the tube by inverting five or six times with the thumb on the top of the tube to prevent the escape of liquid and then withdraw immediately with an ordinary bulb pipette sufficient fluid and allow it to run into one counting chamber. Repeat the operation and fill the other counting chamber.

(8) Count all the cysts in the two separate centimetre squares.

To obtain the number of cysts per gm., the total number in the two squares is divided by 2 and multiplied by 100.

(d) *Stoll counting method* (second modification—centrifuge required)

Apparatus required as for (b).

Method. Steps (1) to (4) are carried out as for (b) and then proceed as follows :—

(5) Fill a centrifuge tube almost to the top with a composite sample of the strained fluid and then spin for two minutes at about 1500 r.p.m. Remove the tube from the centrifuge and then pour off the supernatant fluid.

(6) Agitate the tube until the sediment is loosened and forms a liquid mud at the bottom of the tube and then fill the tube to the same level as before with water.

(7) Thoroughly mix the contents of the tube by inverting five or six times with the thumb over the open end of the tube and then remove 0.15 c.c. of the fluid with the special measuring pipette. Then carry out steps (6), (7) and (8) of (b).

Counting methods for parasites in blood

Sinton (1924) described a method of counting parasites in a fluid by the addition of standard suspensions of fowl red cells in saline to which mercury perchloride is added as a preservative.

In institutions where special apparatus is available counting can be carried out with a special dark ground condenser and an adjustable stop fitted to the 4 mm. objective. A dilution is made in a Thoma hæmocytometer, using a 0.3 per cent. aqueous solution of mercuric chloride as diluent. The usual preparation is made in the counting chamber, the organisms counted and a calculation made to give the number of parasites per c.mm. Trypanosomes show up quite brilliantly under the dark ground illumination even through the thickness of the Thoma counting slide.

Another common technique is to make stained preparations of the blood and then make a direct comparison of the number of parasites and red blood cells.

CULTURE AND MAINTENANCE OF STRAINS OF PROTOZOA

Although protozoa can often be maintained in artificial media and cultural methods may be exceedingly valuable in the detection of infection, the maintenance of fully virulent strains nearly always requires the use of experimental animals. Even in experimental animals many species of protozoa tend to lose virulence unless passage is through the usual vector. Needle-passaged strains can rarely be expected to produce the same clinical picture as strains freshly isolated from the field.

Maintenance of Trypanosomes

(a) *In laboratory animals.* *T. cruzi* is readily inoculable to young mice, rats, rabbits, monkeys and puppies, either by depositing the droppings of infected *Reduviid* bugs on the mucous membrane or by injecting blood from an infected mammalian host. *T. congolense*, *T. evansi*, *T. brucei* and *T. equiperdum* are inoculable to the majority of experimental animals but strains differ in their infectivity to different species and in attempting to establish a laboratory strain it is advisable to use several different species of experimental animal for the first passage. Intraperitoneal inoculation is the most reliable method of infection. The organisms usually appear in the peripheral circulation for a very fleeting period and it is essential that animals should be examined daily after inoculation and that sub-inoculation should be carried out immediately peripheral infection is detected. In subsequent passages in an experimental animal, strains tend to produce more regular and prolonged peripheral parasitaemia. *T. vivax* is normally inoculable only to ruminants but occasional strains have been established in rabbits and guinea-pigs. Desowitz and Watson (1953) described the adaptation of a strain to white rats. *T. simiae* is ordinarily inoculable only from pigs to sheep and occasionally to monkeys. After infection of sheep it is possible to inoculate other experimental animals but the organism tends to revert to the *T. congolense* type and to lose its distinctive morphological characters.

(b) *In culture media.* The different species of trypanosome differ considerably in the ease with which they can be cultured. Non-pathogenic species, particularly those from lower mammals

and some others such as *T. theileri* and *T. cruzi*, are relatively easy to maintain in culture.

T. theileri

Like other trypanosomes of the *lewisi* group this organism can be readily cultivated at temperatures of about 25-28° C. At such temperatures the stages that develop are those which in nature occur in the gut of the invertebrate host and the cultures are not usually viable above 30° C. Simpson and Green (1959) described a cultural method which was in fact used to demonstrate the presence of the parasite. Whole blood was collected aseptically from the animal suspected of being infected and injected into trypticase soy-broth. The supernatant fluid from normal bovine blood that had been hæmolysed with cold distilled water was added. Cultures were incubated at 37° C., growth being made apparent by the appearance of small white dots, strings or flakes on the top of the sedimented layer of blood cells. The colonies became evident after 4-5 days' incubation. Ristic and Trager (1958) obtained cultures of *T. theileri* at 36° C. and 37.5 C. in a blood-lysate medium inoculated with blood from three dairy cows showing subnormal milk production.

Crithidial forms predominated but trypanosomes of the "blood-stream" type, also were numerous.

T. cruzi

This parasite can readily be grown in Novy-MacNeal-Nicolles medium. In cultures grown at 22° C. to 24° C. the trypanosome assumes forms corresponding with the developmental stage in the intermediate host. Little and Oleson (1951) showed that cultures of *T. cruzi* could be propagated continuously in either the liquid or the semi-solid form of a nearly chemically defined medium—supplemented by Difco tryptose. The morphological types of parasite seen included both those seen in the insect vector and in the muscle tissue and the blood stream of the mammalian host.

As a rule the trypanosomes pathogenic to man and domestic animals particularly such species as *T. gambiense* and *T. rhodesiense* are considerably more difficult. They must have the appropriate mammalian serum and blood cells. Such species as *T. brucei* and

T. congolense can be cultured in liquid medium prepared by combining citrated blood with Ringer's solution *e.g.* 1 part blood : 2 parts 75 per cent. sodium citrate solution and 2 parts Ringer's solution (0.6 per cent. sodium chloride). Weinman (1946) described the cultivation of both *T. gambiense* and *T. rhodesiense* and their production in large quantities in a simple cell-free medium consisting essentially of solid blood agar containing inactivated citrated human plasma. Growth could be detected macroscopically by the development of small rounded colourless transparent, slightly raised, glistening areas spotting the surface of the medium. These colonies consisted of large numbers of motile trypanosomes which could be washed off in Tyrode's solution and concentrated by centrifugation.

Trager (1959) maintained tissues of *Glossina palpalis* in culture and was able to get continuous growth of *T. brucei*, *T. congolense* and *T. vivax*. *T. vivax*, previously found to be very difficult to maintain in culture, was grown if:—

(1) Blood was obtained from a sheep with a long-standing chronic infection.

(2) The blood was inoculated into tissue culture two days old.

(3) Incubation was carried out at 30-32° C. *not* 27-29° C.

Morphologically many of the resulting forms were indistinguishable from the classic form of *T. vivax* seen in the proboscis of the tsetse fly but they did not prove infective for sheep. However, a subculture kept at 38° C. did prove infective for sheep.

In general, it is apparent that although cultures of these pathogenic trypanosomes can often be established in blood agar and that good growth can be obtained at 25-30° C. the resulting forms are similar to those of the mid-gut of the tsetse-fly and they do not infect mammals. Tobie (1958) reported successful cultivation of a strain of *T. congolense* for more than a year in diphasic blood agar medium and the appearance of forms definitely "trypanosome" in type with the kinetoplast posterior to the nucleus. Other more stubbly forms probably corresponded with those of the mid-gut of the fly. The strain rapidly became uninfected to white rats. Packhanian (1959) showed that *T. brucei* could readily be cultured in a modified Novy-MacNeal medium provided the particular strain retained its infectivity for tsetse flies. Weinman (1957) has, however, shown that the infectivity to mammals

of *T. rhodesiense* grown in culture may be restored by the addition of *Trehalose* (a disaccharide carbohydrate).

Recent work (Weinman, 1960) has shown that culture may be as good a diagnostic means as any other and may prove useful for the demonstration of cryptic infection in domestic animals. Weinman makes the following points :—

(1) It is particularly necessary to avoid bacterial contamination of the cultures.

(2) Primary isolation tends to need more complicated media than does serial culture.

(3) Strains of trypanosome tend to lose the ability to be cultivated after needle passage through laboratory animals.

An interesting side-light on the culture of trypanosomes has been noted by Lehman (1960) who, in a relatively unsuitable autoclaved N.N. medium, showed differences in growth between *T. rhodesiense* and *T. brucei*—species difficult to separate in other ways.

Culture in eggs

Longley, Clausen and Tatum (1939) showed that *T. rhodesiense*, *T. equiperdum*, *T. brucei* and *T. evansi* could be cultivated in developing chick embryos. It was necessary to subculture every 4-5 days as the embryo died, by transferring diluted blood to a fresh embryo. Chabaud (1939) noted a septicæmia at the seventh to eighth day after which there was a progressive disappearance of trypanosomes from the circulating blood of the embryo. Egg-cultured *T. equiperdum* apparently retains its infectivity for mice (Altire-Werber, 1941). Alwar (1958) cultured *T. evansi* in pigeon and duck embryos, the infection being noticeable in 48-72 hours.

Storage in the cold

Stone and Thompson (1940) showed that *T. equiperdum* could be kept viable and infective for 14 months by storing infected citrated rat blood in an ice bath. Polge and Soltys (1957) showed that trypanosomes cooled to -79°C . and then stored in an insulated chest with solid CO_2 retained their biological characters for ten months.

Maintenance of *Leishmania*

The leishmanias are readily cultivated in various artificial media at temperatures between 22° C. and 25° C., *i.e.* the temperatures at which the sand-fly host normally lives. Novy-MacNeal-Nicolle's medium (N-N-N.) is most commonly used for the culture of both *Leishmania* and *Trypanosoma cruzi*. For experimental and maintenance purposes, dogs, cats, monkeys, mice and rats are all susceptible to experimental infection with *Leishmania* but the hamster is probably the best laboratory animal. Splenic material is the most reliable for the establishment of infection, and intraperitoneal inoculation gives better results than subcutaneous or intravenous. Hamsters remain infected for three to five months, eventually dying with marked œdema. In the case of *L. tropica*, mice can be infected by the inoculation of material into the tail, followed by damage of the tissues at the site of inoculation.

Plasmodium and Babesia

With neither *Plasmodium* nor *Babesia* has culture been successful although organisms can be maintained for limited periods by such methods as have been described by Bass and Johns (1912). With *Babesia* the multiplication of the organisms which occurs in culture is confined to the corpuscles in which the parasites originally occurred. There is no spread to other corpuscles.

Maintenance of strains of *Babesia*

Species of *Babesia* are host-specific and have to be maintained in their natural intermediate or definitive hosts. Ticks often remain infected for several generations even when fed on experimental animals, *e.g.* hedgehogs, in which the parasite cannot develop. In the mammal a heavy infection can usually be best assured in splenectomised animals. In some instances, *e.g.* *B. divergens* in the young calf, splenectomy is essential for clinical symptoms to appear.

Once the infection is established in the mammal under controlled conditions it can be maintained by needle passage or by the use of the usual tick vector. It must be remembered that a needle-passaged strain may develop characters quite different from those of the naturally transmitted field strains. Citrated,

heparinised or oxalated fresh blood is used for sub-inoculation. Blood remains infective for several days, especially if kept cold. Temperatures just above freezing do not seem to have any adverse effect on the parasite.

Callow (1961) showed that the ability of *Babesia bigemina* to multiply so rapidly that it can be found in thin blood smears less than 30 hours after the calf has been inoculated made it possible to separate this parasite by a series of serial passages, at least from *B. argentina*.

Maintenance of Theileria

(a) By needle passage

T. mutans is readily transmitted by the needle passage of citrated blood, the donor remaining infected for years, so there is little difficulty in maintaining the parasite. Transmission of *T. annulata* also is readily achieved as long as the blood is passaged at the height of the temperature reaction. With *T. parva*, inoculation of blood does not usually convey infection. An emulsion of spleen or lymph gland results in a high percentage of infection, but the disease which results is usually much milder than that seen in the field. *T. parva* requires constant passage into fresh animals as in recovered animals the parasites nearly always disappear. Emulsions of ticks which have fed on cattle infected with *T. parva* will often (although not always) set up infection if inoculated subcutaneously into susceptible animals. Alternatively, larval or nymphal ticks from an infected animal, collected and allowed to moult, will usually transmit the infection.

(b) In tissue culture

Tsur (Tchernomoretz) in 1945 and subsequent publications (1953 and 1959) showed that *T. annulata* could be maintained for several days in tissue culture. Growth and multiplication of Koch's blue bodies was observed on rodent spleen tissue. Brocklesby and Hawking (1958) confirmed this work and showed growth for over 59 days. Multiplication of the parasites occurred and the cultures were infective for cattle when tested after 17 and 42 days. These authors, and Tsur, Neitz and Pols (1957) showed also that *T. parva* could be maintained in tissue culture for relatively limited periods. Some multiplication of the parasite

occurred during the first few days but it was confined to the cells of the initial implant. Brocklesby and Hawking (1958) suggested that *T. parva* exerts a toxic effect on cells in tissue culture.

Anaplasma

No cultural methods for either detection of infection or maintenance of strains are available. The organisms can be transmitted by blood inoculation but do not survive long in citrated blood, though when packed in ice, they will survive up to twelve days. The organisms survive longer in defibrinated blood, and Sergeant *et al.* (1945) record that defibrinated blood sent from Algeria to Kenya was still infective after twenty-one days. In cattle, infections are usually complicated by the presence of *Babesia* or *Theileria*, but these organisms can be eliminated by passing the strain through sheep, and then to a clean calf. Cattle infected with *Anaplasma* harbour the organism up to three years, but for the maintenance of strains for immunising purposes, sub-inoculation to new vaccine donors at fairly short intervals is advisable.

Eimeria

After isolation of the oocysts (as described elsewhere) they are allowed to sporulate at room temperatures for a few days, progress of the sporulation being observed with the microscope, and then stored in 2 per cent. potassium bichromate solution. Temperatures from 5° C. to 20° C. are all suitable for storage. Under these conditions oocysts may remain infective for more than a year. Suspensions begin to lose infectivity, however, after about a month.

Entamoeba histolytica

Several different media have been used successfully for the culture of *Entamoeba*, one of the best being Dobell's medium. It must be remembered that *E. histolytica* will not grow except in the presence of a suitable bacterial flora (e.g. *B. coli*).

Maintenance of Histomonas meleagridis

The parasite can be maintained in the laboratory in carrier chickens and a new infection set up in turkeys at intervals with

emulsified fresh cæcal tissue, or it can be kept for several months in the embryonated eggs of *Heterakis gallinæ*. In Britain a high proportion of *Heterakis* eggs seems to be infected.

Two methods are available for infecting turkeys :—

(a) The oral administration of *Heterakis* eggs (at least 1000 eggs should be given to each poult). Clinical disease appears in about fourteen days.

(b) The administration *per rectum* of emulsified infected cæcal tissue. Cæca containing active lesions are removed from a freshly killed bird, placed in a previously warmed mechanical blender and covered with physiological saline at 37° C. Mixing for about thirty seconds is followed by straining through two layers of muslin. The suspension is kept at 37° C. while 1-5 ml. are injected into each bird. The bird is best held in an inverted position for two minutes after injection. Throughout the process care must be taken to avoid chilling the parasite. Clinical disease is apparent in about eleven days.

Maintenance in culture

Histomonas meleagridis can be maintained indefinitely in culture but primary isolation is not always easy and depends on the establishment in the medium of a suitable bacterial flora. The most suitable medium is the diphasic medium of Boeck and Drbohlav.

Lesser (1960) described a number of modified tissue-culture media in which *H. meleagridis* has been grown in the absence of viable bacteria. It appears, however, that heat-labile intracellular factors present in turkey cæcal bacteria are necessary for growth of the parasite.

In vitro cultures of *H. meleagridis*, like many other organisms, lose virulence and will not set up the usual disease in turkey poults.

The preparation of embryonated eggs of Heterakis gallinæ

Collect worms from the cæca of chicks and pour with water through a 30-mesh screen.

Jet worms into a mortar ; pipette off the excess water and dry the worms with blotting paper.

Grind worms with a pestle, pour through a 200-mesh screen,

jetting through with enough water to fill a pint measure. Sediment for at least 3 hours ; siphon off the supernatant fluid and pour the sediment into centrifuge tubes. Spin for two mins. at 1500 r.p.m., tip off the supernatant and add 1 per cent. formalin to the sediment. Pour into 4 in. Petri dishes (about 10 ml. in each) and incubate at 27° C. for twenty-one days. Remove from incubator and store at 5° C.

Trichomonas foetus

The collection of material is described elsewhere. Many different media have been used for the culture of *T. foetus*. One of the best, both for routine culture and for isolation of strains from the field is a bacto-tryptose broth with 2 per cent. dextrose over a slope of inspissated horse serum. An "all liquid" modification is available consisting of glucose bacto-tryptose broth containing 7 per cent. horse serum. For the isolation of parasites from pathological material, a mixture of penicillin and streptomycin should be added to the liquid before inoculation. 100-200 units per ml. of both antibiotics usually is sufficient.

Maintenance of Toxoplasma

(a) *In laboratory animals.* *Toxoplasma* is very readily maintained in laboratory animals and laboratory strains of the parasite are easily passed by any route of inoculation in mice, rats, hamsters, rabbits, guinea-pigs, chick embryos and pigeons. The white mouse is a very satisfactory host and is very rarely found to be spontaneously infected. Rats can carry an experimental infection for at least two years.

(b) *In tissue culture.* The parasite is easy to maintain in tissue culture. A variety of methods has been used, the organism having been cultivated on slides in chick embryo tissue, heart muscle, leg muscle and so on, or in roller culture tubes as reviewed by Jacobs (1956). The yield from a tissue culture is not, however, very big.

(c) *Other methods.* Other ways of preserving *Toxoplasma* have included freezing in glycerine solutions, by yolk-sac passage in the chick embryo, and by inoculating into a hibernating mammal (the marmot).

PREPARATION OF MEDIA

Glucose-broth-serum medium (for culture of *Trichomonas fœtus*)

The medium must be prepared by the following method, paying particular attention to the times and temperatures of sterilisation.

(1) Inspissated serum slopes are prepared in test tubes by heating 4-5 ml. amounts of normal horse serum in a sloping position at 85° C. for thirty minutes.

(2) Bacto-tryptose broth is prepared by dissolving in warm distilled water :—

Sodium chloride	0·5 per cent.
Sodium phosphate	0·25 per cent.
Bacto-tryptose	2·0 per cent.
Glucose	2·0 per cent.

(3) The above liquid is added to the test tubes containing the serum slopes and autoclaved at 15 lb. per sq. in. pressure for ten minutes. Sterile liquid paraffin is finally added to form a thin layer on the surface.

Novy-MacNeal-Nicolle's Medium (N-N-N Medium) (Used for *Leishmania* and *Trypanosoma cruzi* and *T. lewisi*)

This is prepared by dissolving 14 gm. agar and 6 gm. sodium chloride in 900 ml. distilled water, distributing 5 c.c. samples of the solution into test tubes and autoclaving for twenty minutes at 120° C. The tubes are cooled to 50° C. and 15 drops of rabbit blood added to each tube with aseptic precautions. The medium and the blood are mixed gently to avoid the formation of air bubbles and the tubes are placed in a sloping position to set. They are then capped and incubated for 24 hours at 37° C. in order to test sterility and to express the water of condensation. The inoculum is placed in the water of condensation and the tubes are incubated at 24° C. Successful cultures develop in four to seven days.

Media for Trypanosomes

(1) *T. lewisi*, *T. melophagium*, *T. theileri* and *T. cruzi* all show development in defibrinated blood added to broth or to 2 per cent. agar and incubated at 25° C.

(2) *T. brucei* and *T. congolense* have been maintained at 25° C. in a medium consisting of:—

One part blood.

Two parts 75 per cent. sodium citrate.

Two parts Ringer's solution (0.6 per cent. sodium chloride).

(3) *Ringer-Glucose solution*. (This can be used to maintain pathogenic trypanosomes alive for 24 hours at 37° C.)

Sodium chloride	0.9 gm.
Potassium chloride	0.025 gm.
Calcium chloride	0.020 gm.
Sodium bicarbonate	0.015 gm.
Glucose	0.2 gm.
Distilled water	100 ml.

Media for intestinal flagellates

There are three standard media for the culture of these organisms.

The diphasic medium of Boeck and Drbohlav (used for *Histomonas meleagridis*)

Dobell and Laidlaw's modification is prepared as follows:—

The culture tubes contain solid and liquid parts. The solid part consists of serum slopes made by placing 5 c.c. of inactivated horse serum, sterilised by filtering, into tubes, and inspissating them at 80° C.

For the liquid portion a new laid egg is scrubbed with soap and water, treated with alcohol and flamed, and then a hole cut in the broad end with sterile scissors. The white of the egg is allowed to run into a flask containing 250 c.c. Ringer's solution, and after mixing, the solution is sterilised by filtering. A quantity of finely ground rice starch is put into a number of small tubes and sterilised at 180° C. dry heat for one hour. To inoculate cultures fill the egg-Ringer fluid to the top of the serum slope, add a loopful of rice starch and warm the tube to 37° C. Place a loopful of the intestinal specimen at the bottom of the slope. Incubate for 24 hours at 37° C., and then examine by scraping the surface of the base of the slope with a long pipette, and withdrawing some of the debris.

Row's medium

Fill about 30 c.c. of rabbit's blood (obtained with aseptic precautions) into a sterile 100 c.c. flask containing glass beads, and rotate till defibrination is complete. With a sterile pipette add 10 c.c. of the defibrinated blood to a flask containing 90 c.c. sterile distilled water, and when solution is complete, add the mixture to a flask containing 200 c.c. 1.2 per cent. sodium chloride. Distribute 5 c.c. of the solution into test tubes and inoculate with a portion of the suspected material. Incubate overnight at 37° C.

This medium can also be used for *Leishmania*.

Serum saline citrate medium (Cleveland, Andrews and Tanabe)

Dissolve 0.7 gm. sodium chloride and 1 gm. sodium citrate in 100 c.c. distilled water and sterilise in the autoclave. Cool to 50° C. and add 0.5 gm. of Loeffler's dehydrated serum. Add 2 c.c. whipped white of egg obtained with aseptic precautions. Distribute medium into test tubes in 5 c.c. quantities. Inoculate into the bottom of the tubes and incubate at 37° C.

Recent work (Allsop *et al.*, 1961) has suggested that the use of tissue culture monolayers may be a good way of maintaining flagellates and other protozoa from the rumen.

SEROLOGICAL TESTS FOR PROTOZOA

Trypanosomiasis

The complement-fixation test for Dourine. For this test, rats inoculated with *T. evansi* or *T. equiperdum* are killed at the height of infection when trypanosomes are swarming in the blood, and are bled into sodium citrate solution. The citrated blood is centrifuged slowly (2000 revolutions a minute) for five minutes. This throws down the blood corpuscles but leaves the majority of trypanosomes in the fluid. The fluid is pipetted off and then centrifuged rapidly (10,000 revolutions a minute) for ten minutes, the trypanosomes being thrown down as a white deposit. These are separated from the fluid, washed by centrifuging several times with saline and are then emulsified in Watson's preservative (normal saline 9 parts, pure neutral glycerin 1 part, formol 0.1), and maintained at 0 to 4° C., at which temperature this antigen will keep three months or more. In hot climates samples of blood

sent in for testing do not keep satisfactorily in transit, and it is found better to draw a few cubic centimetres of blood, allow it to clot, draw off the serum with a pipette, and add one-tenth the quantity of 5 per cent. phenol in glycerin before sending the sample for examination. The antigen is titrated with a known positive serum. Horse serum can be inactivated at 58°C ., but donkey serum is anticomplementary and should be inactivated at 65°C .

For the test a sample of serum is tested against the antigen, using negative and positive sera controls ; in the case of the latter, in addition to the normal antigen dilutions, tubes are also included at double and half the normal strength of the antigen.

If the serum under examination gives a positive reaction, it is then re-tested in four dilutions to obtain a titre.

French authorities claim that this formol-glycerin antigen has poor keeping qualities, and recommend an antigen prepared by centrifuging infected rat's blood at great speed for half an hour, washing the sediment, mainly leucocytes and trypanosomes, in distilled water to remove any remaining erythrocytes, and then in saline. The remaining sediment is then taken up with acetone, the acetone is evaporated in an incubator, and the residue taken up with absolute alcohol (1 c.c. of residue to 10 c.c. alcohol) and kept in hermetically sealed coloured glass phials. For use the alcohol is evaporated and the residue dissolved in saline (five times as much saline as the original quantity of alcohol). This antigen is said to keep up to three months. Zottner (1934).

The mercuric chloride test for camel surra

One drop of serum from a suspected camel is added to 1 c.c. of a 1 : 25,000 aqueous solution of mercuric chloride. Sera from infected animals give a white precipitate which is not produced by sera from normal animals. The test has been used on a large scale in the Sudan in measures for the control of surra, and has given very satisfactory results (Bennett, 1928 and 1933). Positive results are, however, also given by camels infected with *T. vivax*.

Leishmaniasis

Formol-Gel test for Leishmaniasis in dogs

One drop of formalin is added to 1 c.c. of serum, the result being judged by the rapidity of gellification and the appearance

of opacity. In infected animals gellification should occur within an hour, the gel becoming opaque with standing. The test is not absolutely reliable, but animals giving positive readings should be treated as suspects, even if other evidence of infection is not obtained. A precipitation test for canine leishmaniasis, using urea stilbamine, neostilbamine and distilled water has been described.

Anaplasmosis

Boynton and Woods (1935) reported a non-specific test which has some value. Blood is drawn, allowed to clot and the clear serum separated. When two drops of the serum are added to 2 ml. of distilled water there is an immediate cloudiness with later precipitation. The serum of normal animals does not become cloudy in water.

The complement-fixation test for Anaplasmosis

This has been discussed by Gates and Roby (1956).

Toxoplasmosis

Three types of test are available :—

- (1) The dye test of Sabin and Feldman
- (2) The complement-fixation test
- (3) Neutralisation tests (usually in tissue culture).

The dye test

This test, described by Sabin and Feldman in 1948, depends on the fact, that when toxoplasms are stained by alkaline methylene blue in the presence of normal serum they have a characteristic morphology and staining reaction. In the presence of specific immune serum the organisms are distorted and the cytoplasm does not take up the stain. The sensitiveness of the test depends on the sample of methylene blue used and there is the possibility of non-specific reaction. In particular, a positive Sabin-Feldman reaction may be obtained with animals infected with sarcosporidia.

The complement-fixation test

This is not as generally useful as the dye test. Complement-fixing antibodies appear later in the course of the disease than those detected by the dye test and disappear rather rapidly with time. Antigen may be prepared in a variety of ways. Chorioallantoic membrane or tissue culture are probably best.

Non-specific reactions may occur. There is a cutaneous test using "Toxoplasmin" but a negative test does not necessarily imply absence of antibody. The method is described by Frankel (1948).

Cathie (1957) gives an appraisal of the relative merits of the tests available for the detection of *Toxoplasma*. A combination of the dye-test and the complement-fixation test is best.

The agglutination test for *Trichomonas foetus*

The basis for these tests has been described by Pierce (1947 and 1949a). In a proportion of cases sera from infected animals give a positive reaction. In addition, agglutinins to *T. foetus* may be found in the vaginal mucus of infected cows, particularly in animals suffering from pyometra or in other infected animals during the four to five days following œstrus. It is necessary to carry out all diagnostic tests in duplicate using the two strains, *Manley* and *Belfast*.

The mucus agglutination test

The mucus is collected, brought to the laboratory and kept in the refrigerator until tested.

The following equipment is required :—

1. 10 c.c. of hot nutrient agar.
2. Five plugged test tubes $6 \times \frac{5}{8}$ in.—sterile and washed with glass distilled water.
3. Four small Petri dishes $1\frac{1}{2}$ in. in diameter—sterile and washed with glass distilled water.
4. One sterile pipette.
5. One Griffiths tube.
6. Three sterile 10 c.c. pipettes.
7. One culture tube of *T. foetus* Manley—3 day growth.
8. One culture tube of *T. foetus* Belfast—3 day growth.
9. 160 c.c. of saline.
10. 80 c.c. glucose broth ($2\frac{1}{2}$ per cent. glucose with 1 per cent. peptone in nutrient broth).

1 to 5 are required for each test. 6 to 10 will be sufficient for forty tests.

Method

1. Emulsify each mucus sample in turn in a Griffith's tube with saline using 2 c.c. of mucus to 8 c.c. of saline. Pour

into a test tube, replug and number. The Griffith's tube must be clean but need not be sterile.

Rinse the Griffith's tube under the tap and repeat for each test.

2. Label Petri dishes and tubes, M 1/10, M 1/20, B 1/10, B 1/20.

We now have a series of five tubes, the first containing the diluted mucus and the others labelled in order as above. The tubes in order are :—

1	2	3	4	5
contains mucus dilution	empty labelled M 1/10	empty labelled M 1/20	empty labelled B 1/10	empty labelled B 1/20

3. Two c.c. of saline are placed in tube 3 and 2 c.c. of emulsion are placed in tubes 2, 3 and 4. The emulsion and saline in tube 3 are mixed thoroughly, 2 c.c. withdrawn and placed in tube 5.

This process is repeated with each mucus sample being tested.

4. Now add 2 c.c. agar to tube 2, shake well to mix and pour into the corresponding Petri dish. Repeat for tubes 3, 4 and 5 ; the same pipette can be used for all.

Leave all to set whilst preparing the antigen.

5. *Antigen*. Mix 160 c.c. of saline with 80 c.c. broth and divide into two. To the first half add a culture of Belfast strain, shake and label. Repeat with second half using Manley strain. Examine the tubes and check the contents. There should be about 100 Trichomonads per c.mm., *i.e.* 100,000 per c.c.

Pipette 1½ c.c. of the appropriate suspension into each dish, using a separate pipette for each strain.

6. Incubate 1-1½ hours at 37° C.

7. Leave shaded on the bench ½ to 1 hour and read under the low power of the microscope focusing on the surface of the agar. The agglutination is assessed according to the scale given below. An agar+ suspension control is included with each test.

Reading the test

The agglutination results produced by different concentrations of antibodies are recorded as described below.

+++++ . A pre-agglutination (P.A.) characterised by immobilisation, with an absence of clumping, and a change in the appearance of the organisms. Small aggregations into flat leaf-like clumps may also be observed.

- +++++ . An echelon leaf-like clumping (E) with complete immobilisation (+++++) or with much reduced motility (+++++).
- ++++ . Big clumps, very compact, with the flagella of the organisms slightly active.
- +++ (+) . Big round clumps of organisms with active flagella round the periphery.
- +++ . Round clumps of *active* organisms with a few free unagglutinated organisms.
- ++ (+), ++ . Descending degrees of agglutination, with increasing mobility and a gradual increase in the number of unagglutinated organisms.
- + . . A dispersed culture with occasional moderate-sized clumps and small aggregations of a few active organisms, many unagglutinated organisms.
- (+) . . A dispersed culture with very small active clumps, aggregations of a few active organisms loosely attached by their flagella, very many unagglutinated organisms.

A +++ (+) result given by a 1/20 dilution of mucus in this test is indicative of the presence of infection. It should always be confirmed by demonstrating the presence of the parasite.

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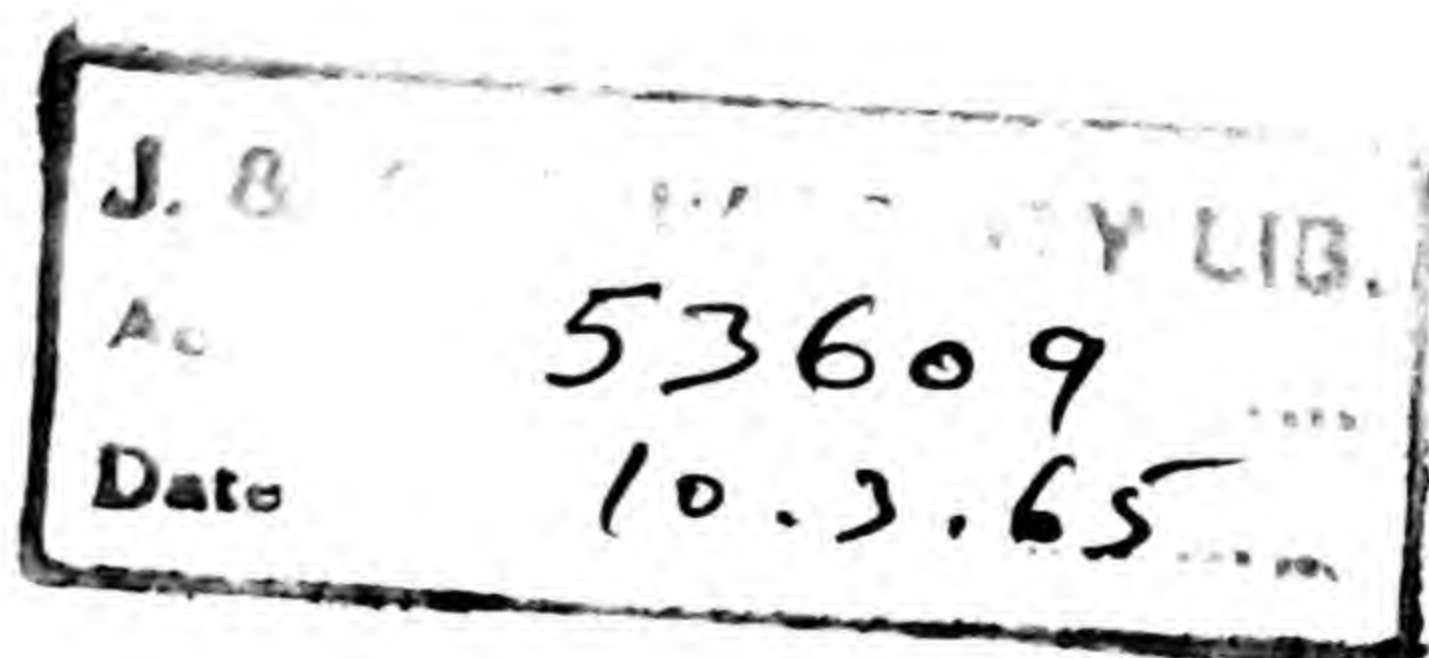
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